DESIGN, SYNTHESIS, CHARACTERIZATION AND INVITRO BIOLOGICAL EVALUATION OF NOVEL SERIES OF 1- SUSTITUTED ISATIN DERIVATIVES BY MANNICH REACTION

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai

In partial fulfilment of the requirements for the award of the

Degree of

MASTER OF PHARMACY

MARCH – 2014

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
COLLEGE OF PHARMACY
MADURAI MEDICAL COLLEGE
MADURAI – 625 020.

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CERTIFICATE

This is to certify that the dissertation entitled – **DESIGN SYNTHESIS, CHARACTERIZATION AND INVITRO BIOLOGICAL EVALUATION OF NOVEL SERIES OF 1- SUBSTITUTED ISATIN DERIVATIVES BY MANNICH REACTION** was done by **Mr.K.SASIKUMAR (Reg.No.261215754)** in the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai- 625020, in partial fulfillment of the requirement for the Degree of Master of pharmacy in Pharmaceutical chemistry under guidance and supervision of **Prof. (Mrs.) R.THARABAI, M.Pharm., HOD,** Department of Pharmaceutical Chemistry in the academic year 2013-2014.

The dissertation is forwarded to the Controller of Examination, The TamilNadu Dr.M.G.R. Medical University, Chennai.

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Date :

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Date:

Evaluation Certificate

Internal Examiner

External Examiner

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Dedicated to

My Family Members,

Teachers, Friends

and

My Well Wishers

CONTENTS

DETAILS OF ABBREVIATION

°C	:	Degree Centigrade
gm	:	Gram
mg	:	Milligram
mol	:	Mole
Ar	:	Aromatic
Rf	:	Retention factor
Str	:	Stretching
mm	:	Milli meter
M.wt	:	Molecular weight
M.F	:	Molecular formula
DMSO	:	Dimethyl sulfoxide
%	:	Percentage

INTRODUCTION

MEDICINAL CHEMISTRY:-

The Medicinal chemistry is the Branch of Science, which study about the synthesis, SAR, QSAR, Molecular biology, structure modification for optimization of their activity and Biological activity of medicinal compounds. Drug synthesized from natural source by Extraction and isolated the lead compounds. Then it is involved in the semi synthetic, synthetic process and retro synthetic analysis.

Medicinal chemistry gives new strategies in the field of Drug research, it scrutinised the physiochemical properties, Drug receptor binding mechanism and computer aided Drug Design of medicinal compounds. Recently Drug discovery studies have focused on the design and synthesis of small molecules combine to form new drug, it is emphasised in combinatorial chemistry.

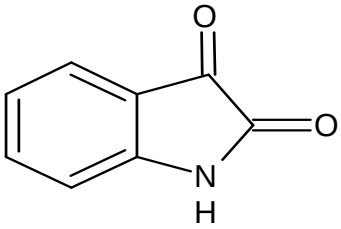
Combinatorial chemistry can be viewed as a tool which allows large number of compounds to be synthesised simultaneously in a time taken to prepare only handful compounds by traditional Synthetic methods.

Medicinal chemistry is one of the most rapidly developing areas within the discipline of chemistry both globally and locally. It is the study of the design, biochemical effects, regulatory and ethical aspects of drugs for the treatment of disease.

Medicinal chemistry combines to form a set of highly interdisciplinary sciences, setting its organic physical and computational emphases alongside biological areas such as biochemistry, molecular biology, Pharmacognosy and pharmacology, toxicology and human medicine; these with project management, statistics and pharmaceutical business practices, systematically oversee altering identified chemical agents such that after pharmaceutical formulation, they are safe and efficacious and therefore suitable for use in treatment of disease.

ISATIN AN OUTLOOK[6]

BASIC NUCLEUS



Isatin or 1H-indole 2, 3 Dione is an indole derivative. The compound was first obtained by Eradman and Laurent in 1841 as a product form the oxidation of indigo dye by nitric acid and chromic acids. The compound is found in many plants such as Isatistinctoria, Calanthediscolor and in Couroupitaquianensis.

Structural activity relationship (SAR)

- Bond acceptor at the position 3
- Free rotation bond O≠ H
- Bond donar at the position 1
- Polar surface area 37,38
- C5, C6 and C7 substitution generally enhanced CNS activity with some Di and tri halogenated isatin

Isatin [1H-Indole-2,3-dione] an oxidised derivative of indole, was first Discovered by Erdmann and laurent in 1840 as a product arising from the oxidation of indigo using nitric acid and chromic acid. The compound was considered synthetic for almost 140 years until it was found to be present in plants from the Isatis genus, in fruits of the cannon ball tree, couroupita guianensis aubl and in secretion from the parotid gland of the Bufo frog. Various substituted isatins have also been identified in plants, fungi, Symbiotic bacteria and marine molluses. Where they are postulated to play a defensive role against pathogenic organism.

In humans and other mammals, isatin is found as an endogenous molecule. Although the metabolic pathways of isatin have not yet been fully elucidated, it has been proposed that it is synthesised in vivo from tryptophan – rich foods such as meat, dairy and whole grains. In this pathway ,tryptophan is converted to indole by bacteria from the gastro intestinal tract and then transported to the liver .where it is oxidised. Isatin also plays major role in many physiological pathways.

Isatin is a versatile chemical building block, able to form a large number of hetero cyclic molecules. The compound possesses an indole ring structure , common to many pharmaceuticals, isatin itself possesses an extensive range of biological activities. Isatin is able to participate in a broad range of synthetic reactions, leading to extensive use as a precursor molecule in medicinal chemistry. Here we discuss the potential of isatin and it derivatives to create novel bioactive compounds. The basic chemistry and synthesis of isatin derivatives are first reviewed. Expanse of biological, and particularly pharmacological activities of isatin compounds are explored. During this discussion, we propose molecular modification to tune and refine isatin compounds for use in specific therapies.

Isatin and its derivatives are responsible for a broad spectrum of biological activities. Among these the cytotoxic and anti neoplastic properties have been the most widely reported. The synthetic versatility of the isatin, due to its privileged scaffold, has led to the generation of a large number of structurally diverse derivatives which include analogues derived from either mono, Di, tri substitution of the aryl ring A, and/or those obtained by derivatisation of the isatin nitrogen and C2/C3 carbonyl moieties. These compounds inhibit cancer cell proliferation and tumour growth via interaction with a variety of intracellular targets such as DNA , telomerase , tubulin , p-glycoprotien , protein kinases and phosphatases. Here in we review recent highlights in the development of isatin based compounds as anticancer agents with a particular focus on the cyto toxicity and structure activity relationship.

ANTI OXIDANT[11,12]

Anti oxidants are chemical substances that donate their own electron to free radicals thus preventing the cellular damages. Anti oxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates they exert the effects by scavenging reactive oxygen species [ROS] or preventing the generation of [ROS].

Naturally there is balance between the amounts of free radicals produced in the body Anti oxidants. The amount of anti oxidants in normal physiological condition is insufficient to neutralise free radicals generated in disease or ill condition. Therefore it is obvious to enrich our diet with anti oxidant to protect from harmful disease.

Oxidative stress is defined in general as excess formulation and are complete removal of highly reactive molecule such as oxygen species, singlet oxygen O2* superoxide anion [$O2^{-}$] peroxide radical[RO2] α hydroxyl radical [OH] are through to cause oxidative damage .Oxidative stress further leads to ageing process and degenerative disease like cancer, inflammation, cardio vascular and Neurodegenerative disease.

INVITRO MODELS OF ANTI OXIDANT STUDIES

1] Conjucated diene assay

2] DPPH method

3] Superoxide radical scavenging activity

4] Hydroxyl radical scavenging activity

5] Nitric acid radical inhibition assay

6] Reducing power method

7] Phospo molybdenum method

8] Peroxy nitrile radical scavenging activity

9] N,N dimethyl P- pheylenediamine dihydro chloric method

10] Oxygen radical absorbance capacity

11] B-carotene oxidase method

12] Xanthene oxidase method

13] Ferric reducing ability of plasma

14] Total radical trapping antioxidant potential

ANTI NEOPLASTIC ACTIVITY[26,27,28]

Anti Neo plastic agents are the drugs used in the treatment of cancer, malignancy, tumour, carcinoma, sarcoma, leukaemia, etc. Cancer is a class of disease or disorder characterized by uncontrolled division of cells and the ability of these to spread either by direct growth into adjacent tissue through invasion or by implantation into distant sites by metastasis.

Two key aspects of cellular life are

1] DNA synthesis and mitosis to produce new cells

2] Cell differentiation which Produce specialised cells.

Normally cell have control mechanism to moderate these two process by growth factor or growth inhibitors A balance between cell growth and cell death is maintained. Cell death is actively regulated by process known as apoptosis. Apoptosis is defined as a cell process of cell shrinkage, membrane blabbing and nucleus condensation.

In cancer cell this regulatory process is aberrant they produce over production of growth factor and avoid apoptosis which continue to multiply in an unregulated manner. The unregulated growth causes damage to DNA, resulting in mutations to genes that encode from protein controlling cell division.

Cell cycle and regulation

The cell cycle is divided into four parts

1] G1 or Gap 1,

2] G2, M and S phase

G1—the G1 phase is the period when newly created cell is born

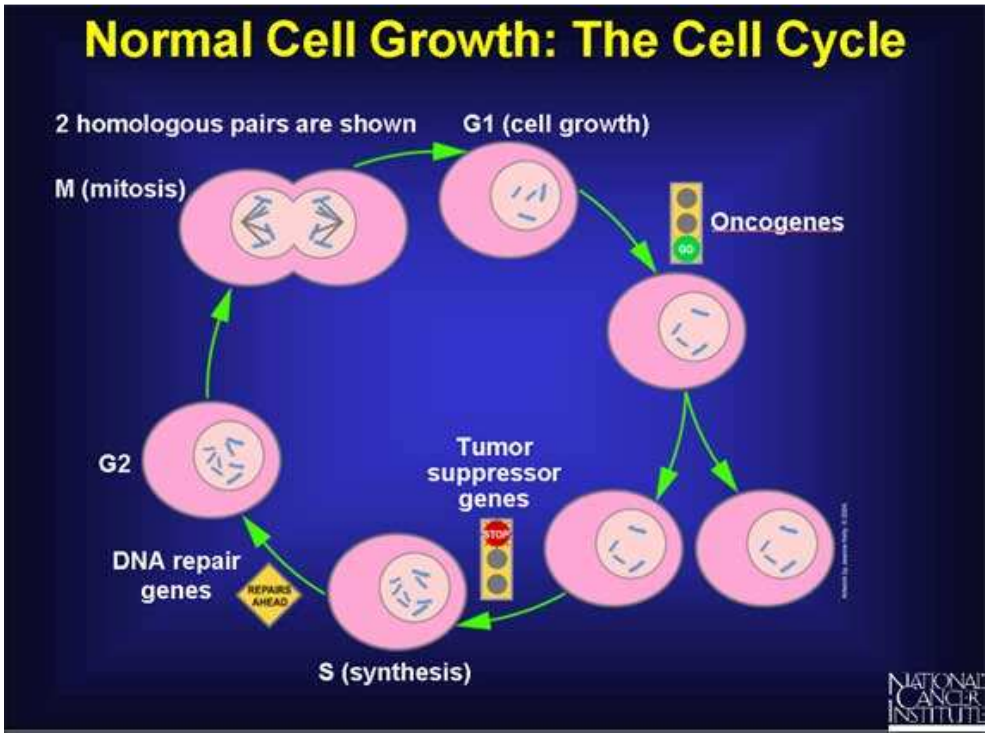
S – Synthesis phase , in this period DNA replicates and two copies of DNA are present inthe Cell.

G2 – G2 phase and cells are prepared for final cell cycle phase M phase

M phase - mitosis or cell division

G1/S phase is important in understanding cancer and cancer treatment.

The cell cycle is controlled by water soluble protein called growth factor and binds to glycoprotein receptors. The growth factor autocrine controls the number of cell in either proliferated or non proliferated state and thus maintain homeostatic many cancer cells produce excessive level of this growth factor.



ANTIBACTERIAL ACTIVITY[50]

Antibacterial agent: The drug which inhibit or destroy the growth of bacteria

Organism used for activity:

Salmonella Typhi:-

1) Gram negative bacteria
2) Motile facultative anaerobe
3) Responsible for typhoid
4) Possible to control by proper hygiene

Staphylococcus Aureas:-

1) Gram positive bacteria
2) Round shape and golden yellow colour
3) Responsible for sinusitis, skin disease
4) Possible to control by proper hygiene

Mechanism:-Anti Bacterial agent inhibit

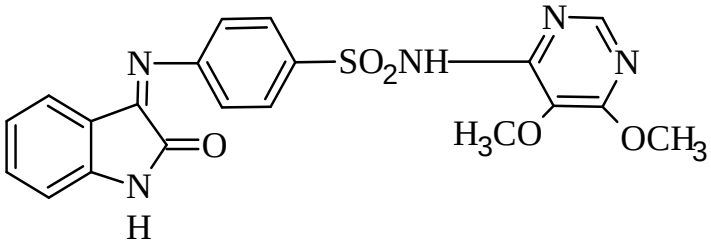
1) Cell wall synthesis
2) Protein synthesis
3) Enzymatic activity
4) Folate metabolism

Evaluation method :-

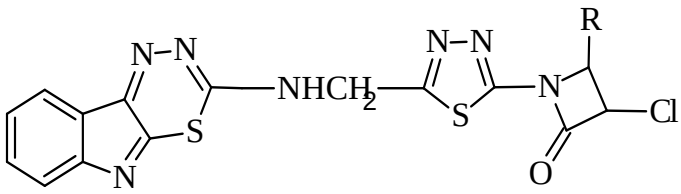
1) Turbidimetric method
2) Agar cup plate method

LITERATURE REVIEW

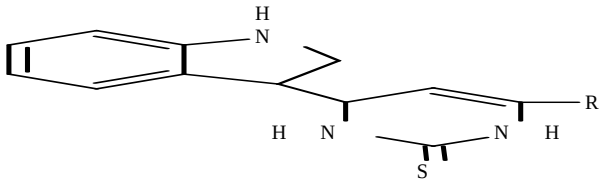
- **PANDEYA *et al.,*** synthesized Schiff bases of isatin and5-methyl isatin with sulphadoxine. The synthesized compounds were tested for antimicrobial activity.



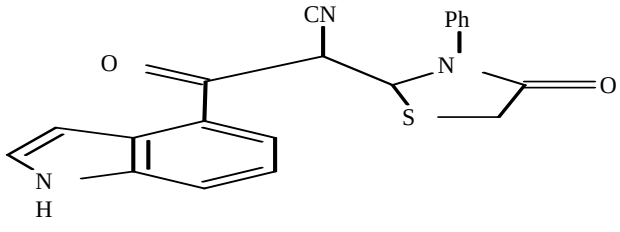
- **KUMAR *et al.,*** synthesized a series of new substituted azetidinoyl and thiazolidinoyl-1,3,4- thiadiazino (6,5-b) indoles and tested for anti inflammatory activity.



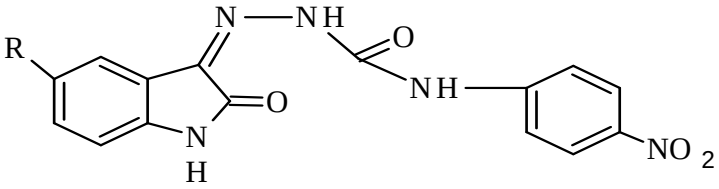
- **AMIR *et al.,*** prepared and screened a series new indole derivatives with anti inflammatory activity.



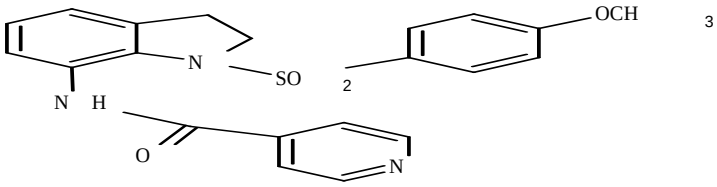
- **RADWAN *et al.***, synthesized and evaluated the analgesic activity of 3-substituted indolederivatives.Thethiazolidine -4-one derivatives was found to be exihibit analgesic activity.



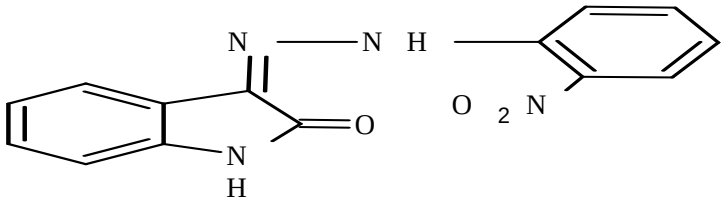
- **PANDEYA *et al*.,** synthesized a series of p-nitrophenylsuntitutedsemicarbazone and evaluated for anticonvulsant activity.



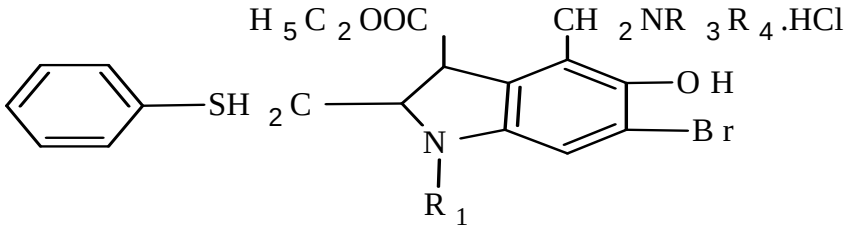
- **LIOU *et al*.,**synthesized and evaluated a series of new indoline-sulfonamide with anti cancer activity.



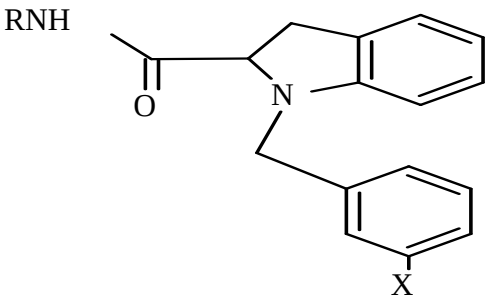
- **POPP and PAJOUHESH *et al*.**, synthesized 3-o-nitrophenylhydrazones of isatin by condensation of isatin with o-nitrophenylhydrazine. These compounds were tested for anticancer activity.



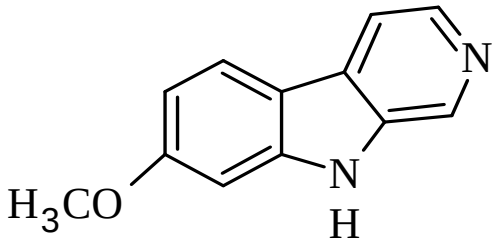
- **DUNWANG *et al*.,** synthesized some new derivatives of 3-ethoxycarbonyl-6-bromo-5-hydroxy indole and evaluated for antiviral activity.



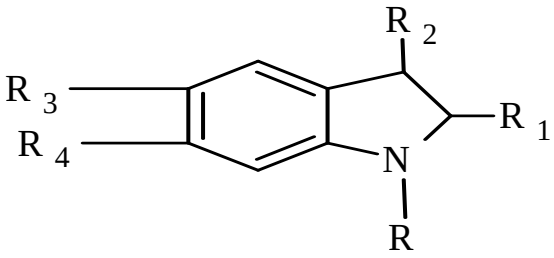
- **ENIEN *et al.,*** synthesized indole-2 and 3-carboxamide and evaluated their biological activities as antioxidant activity.



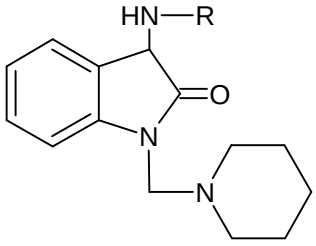
- **MISHRA *et al.,*** presented a review on natural products as antileishmanial and showed the Harmaline is an indole alkaloid as potent leishmanial agent.



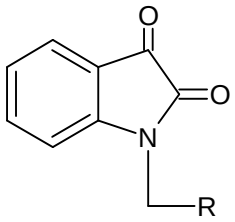
- **CHAUDHARY *et al.,*** showed that various indole derivatives act as effective antifertility agents.



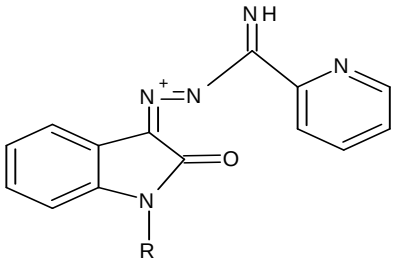
- **Jnyanaranjan Panda *et al,*** reported on the synthesis of Mannich based novel 1, 3-disubstitued Isatin derivatives of Antibacterial and Antifungal agents.



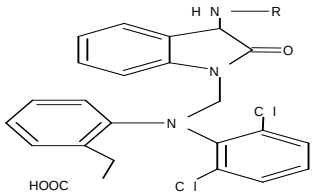
- **Khalaf Ahmed Jasim*et al*,** submitted the study of some new Mannich bases derived from Isatin (1H-indole -2, 3 –Dione) for Antimicrobial activity.



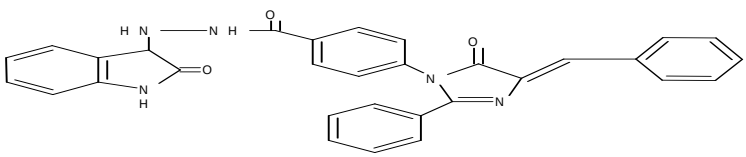
- **B. vijaya Kumar *et al*,** reported on the design and synthesis of novel pyridyl – 2- amidrazone incorporated Isatin Mannich bases for Antimicrobial activity.



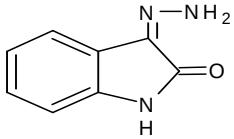
- **V. Ravichandran*et al*,** reported on the synthesis of Mannich bases of Isatin and its derivatives with 2-[(2, 6 – dichloro phenyl) amino] phenyl acetic acid for Antimicrobial activity.



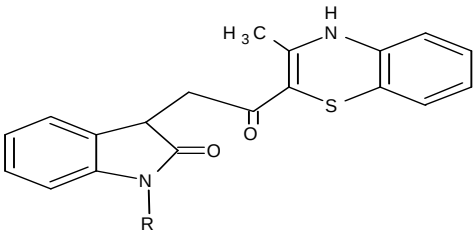
- **Sanjay Bari *et al*,** reported on the synthesis of 3-[(5-benzylidene -2-nones for their Antimicrobial activity.



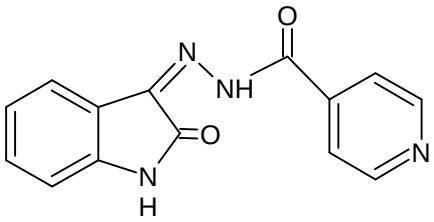
- **Madhu*et al*,** reported on the synthesis of [3-hydrazono -1, 3- dihydro – 2H – indole -2-one] for their Antimicrobial activity.



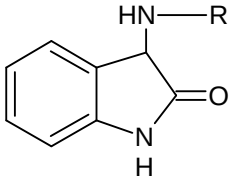
- **Dhananjay More *et al*,** reported on the synthesis of 1,4 – benzothiazine compound containing Isatin moieties as Antimicrobial agent.



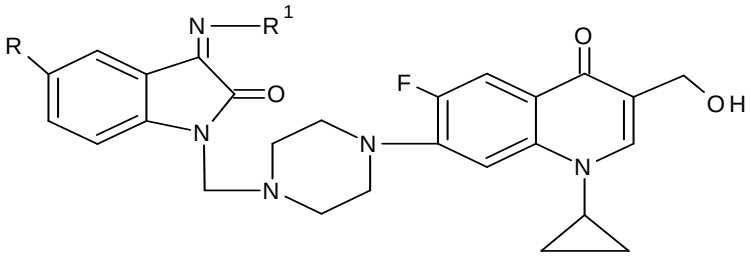
- **Yifeng Sun *et al*,** reported on the synthesis and crystal structure of isatin -3-isonicotinoyl hydrazine as Antibacterial agent.



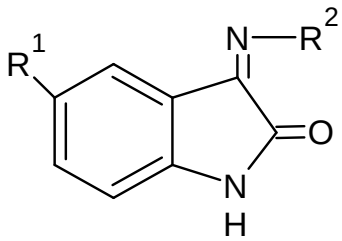
- **Jnyanaranjan Panda *et al*,** reported for efficient synthesis of Isatin derivatives and evaluation of their Antibacterial activities.



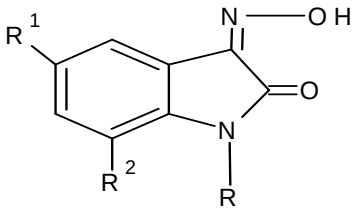
❖ **Ramachandran.S *et al,*** reported on the synthesis of Mannich based novel 1, 3, 5 trisubstituedIsatin derivatives for Analgesic and Ulcerogenic evaluation.



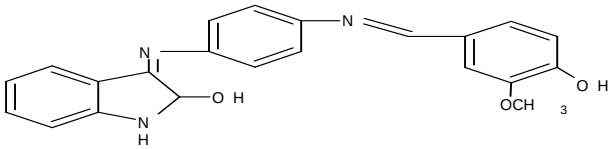
❖ **Nadeem Siddiqui *et al,*** synthesized novel 3, 5 – disubstituedIsatin derivatives of Anticonvulsant activity.



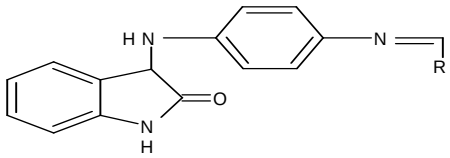
❖ **SurendraNathPandeya*et al,*** reported on the synthesis and Anticonvulsant activity of substituted Isatin -3-oximes.



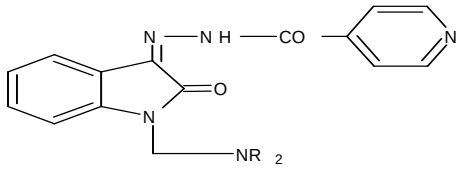
❖ **Vinit Raj *et al,*** review on Anticonvulsant activity of Isatin derivatives.



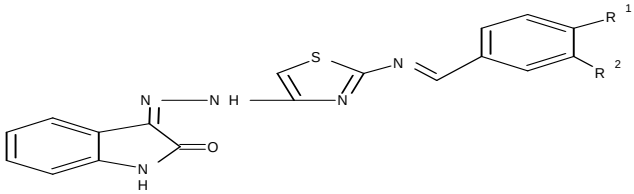
- **Chinnasamy Rajaram Prakash *et al,*** reported on the synthesis characterization and Anticonvulsant activity of novel Schiff base Isatin derivatives.



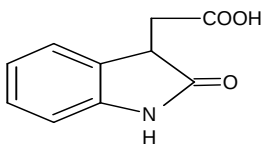
- **Mostafa.A.Hussein*et al,*** synthesized some Mannich bases derived from Isatinisonicotinic acid hydrazone for Antitubercular activity.



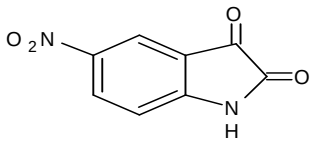
- **Venkateshwar Rao *et al*,** reported on the synthesis of isatin -3- [N2-(2-benzalamino thiazol-4-yl)] hydrazine for their Anti-inflammatory activity.



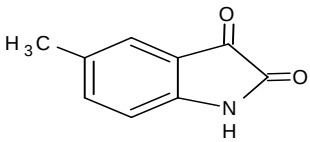
- **TarekAboul – Fadl*et al,*** synthesized methisazone plays an important role as prophylactic agent against several viral diseases
- **A.W.Galston *et al,*** reported on the synthesis of oxindole -3- acetic acid for auxin activity.



- **Ratnamala.P.Sonawane*et al,*** reported on the synthesis and spectral studies of substituted 5- Nitro-1H-indole-2, 3-dione.



- **JiroTatsugi *et al,*** reported on improved preparation of Isatin for indoles.



AIM AND OBJECTIVES[8,10]

Isatin and its derivatives are responsible for a broad spectrum of biological activities. Already many of the scientist has done the cytotoxicity studies from isatin derivatives. Which is substituted by various molecule in the position of isatin certain nitrogen atom,C2,C3 carbonyl moieties and mono, Di, tri substitution of aryl ring A.

In this way I would like to substitution the various aromatic secondary amine in the position of isatin containing nitrogen by mannich reaction and also I have tried with various aldehyde instead of formaldehyde for the cytotoxicity studies and anti bacterial studies. Cytotoxicity studies are carried out through cervical cancer cell line.Anti bacterial activities are studies through cup- plate method. Anti oxidant properties are studied by hydrogen peroxide method.

OBJECTIVES:

To synthesis isatin derivatives by mannich reaction by using stirring method. It is synthesized by two steps .

a) Isatin synthesis from aniline with chloral hydrate by sandmayer reaction.
b) Isatin react with various aromatic secondary amine like piperzine, Diphenyl amine, pyrolidine, benzimidale by mannich reaction.

The synthesized novel series of isatin derivatives were characterized by thin layer chromatography and spectral studies like NMR, MASS, IR spectroscopy.

Isatin derivatives are designed by following software1

a) CHEMDOODLE

b) MOLNSPIRATION

c) CHEM SKETCH

Biological evaluation of isatin derivatives

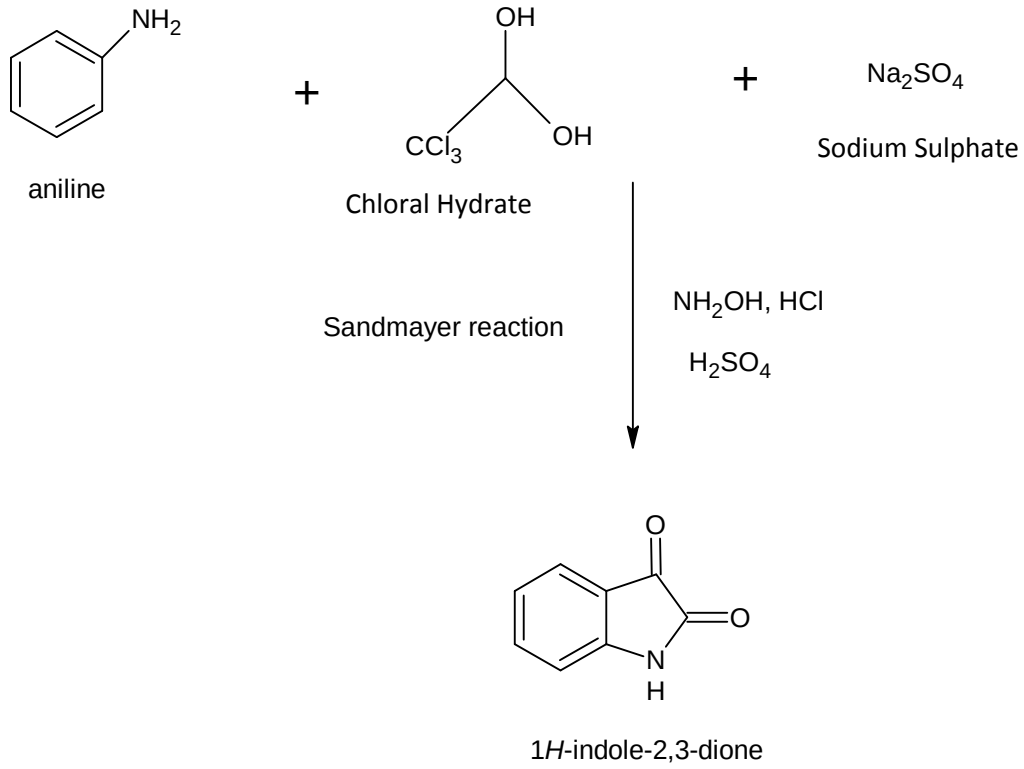
a) Invitro studies of anti oxidant properties

b) Invitro studies of anti bacterial activity

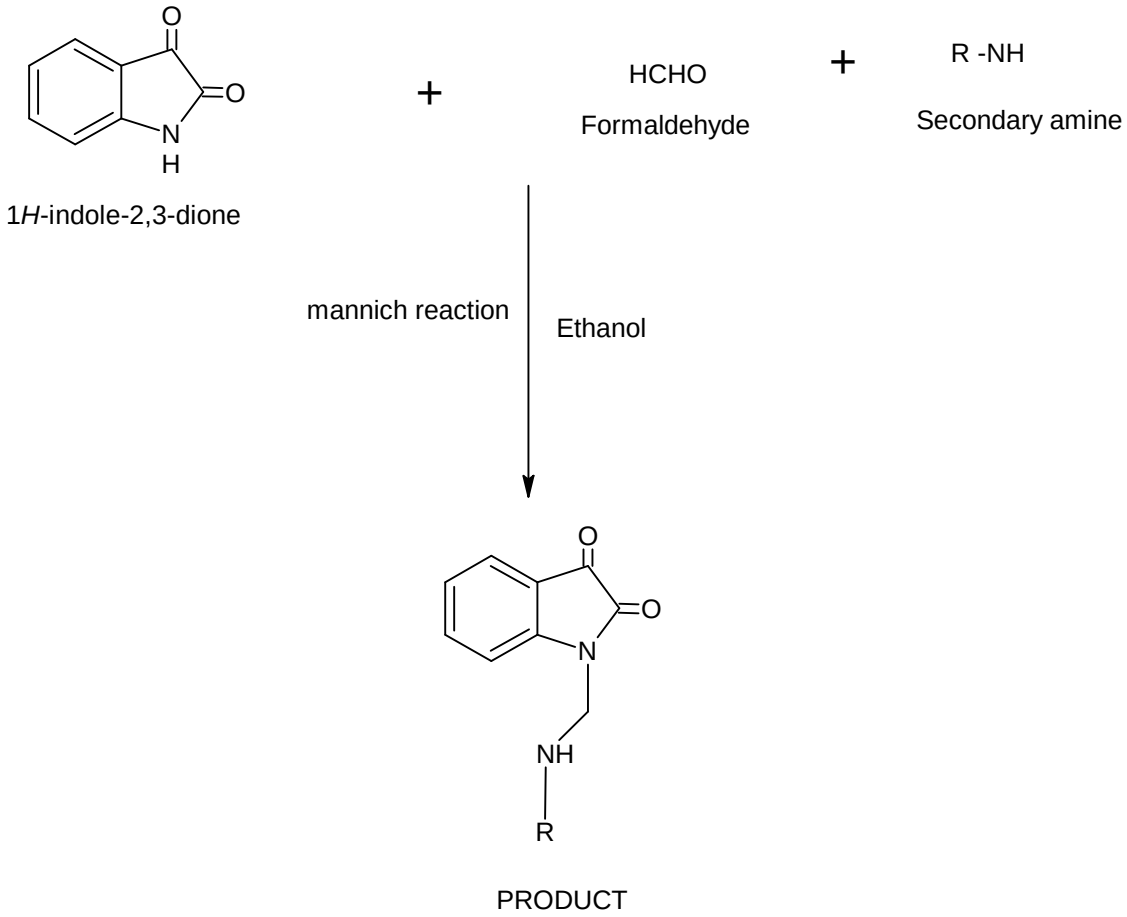
c) Invitro cytotoxicity studies against cervical cancer cell line.

SCHEME OF REACTION

SCHEME : 1



SCHEME : 2


O
O
N
H
1*H*-indole-2,3-dione
+
HCHO
Formaldehyde
+
R -NH
Secondary amine
mannich reaction
Ethanol
O
O
N
NH
R
PRODUCT

EXPERIMENTAL PROCEDURE

STEP 1

Synthesis of -1H-indole-2,3-dione(isatin)

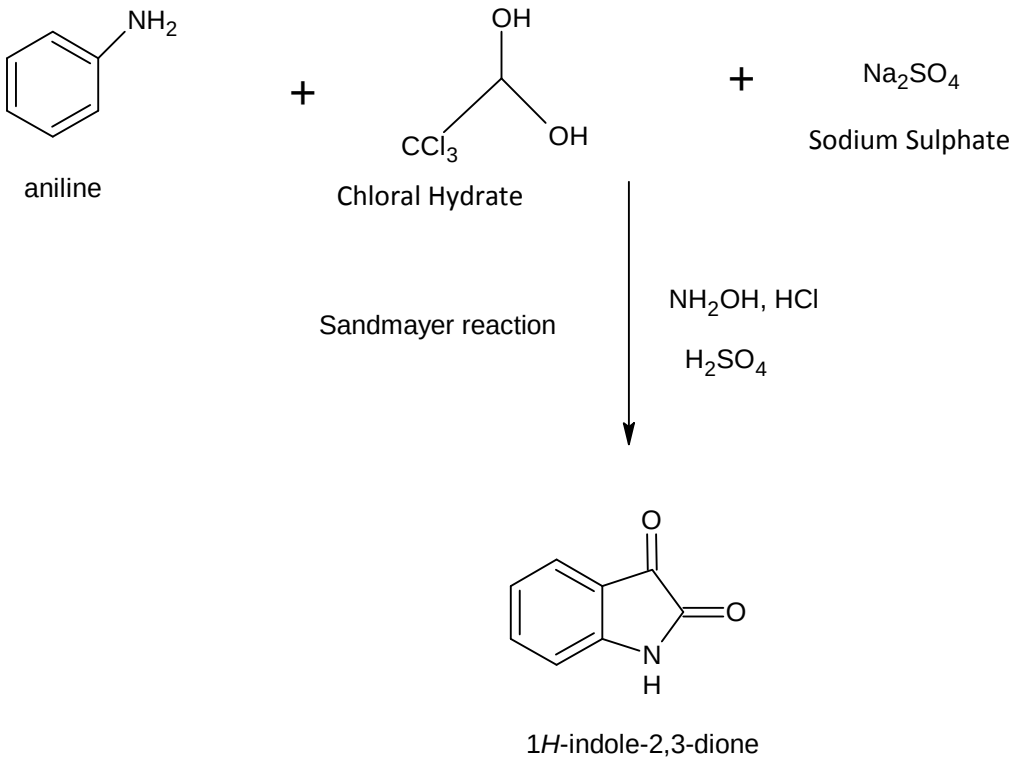
Chemicals required:

- Chloral hydrate
- Aniline
- Sodium sulfate
- Concentrated hydrochloric acid
- Hydroxylamine hydrochloride
- Water

Procedure:

Dissolve chloral hydrate, sodium sulfate and aniline in water. To this add concentrated hydrochloric acid and hydroxylamine hydrochloride and heated on a water bath for about 40-50 minutes. The dried sample is obtained to this add warm concentrated sulphuric acid and stirred it in a temperature between 60-70c. until the reaction get completed. Then it is cooled and washed with cold water and dried in air.

Synthesis of 1H-indole-2,3-dione



COMPOUND S1

Synthesis of 1(piperazine-1-ylmethyl)-1H –indole-2,3 dione

Chemicals required:

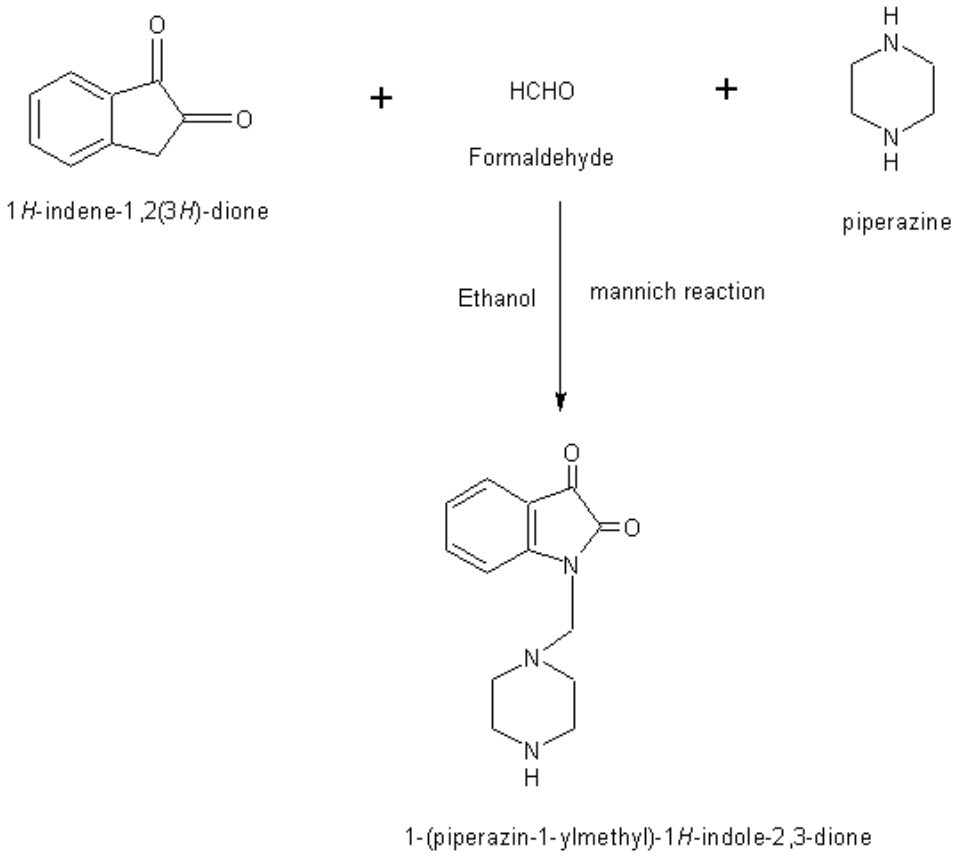
- Isatin -2mmol
- Piperazine -2mmol
- Formaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and formaldehyde 37% (3mmol) and add Piperazine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene: Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S1

Synthesis of 1(piperazine-1-ylmethyl)-1H –indole-2,3 dione



COMPOUND S2

Synthesis of 1-(phenyl(piperazine-1-yl)-H-indole-2,3 dione

Chemicals required:

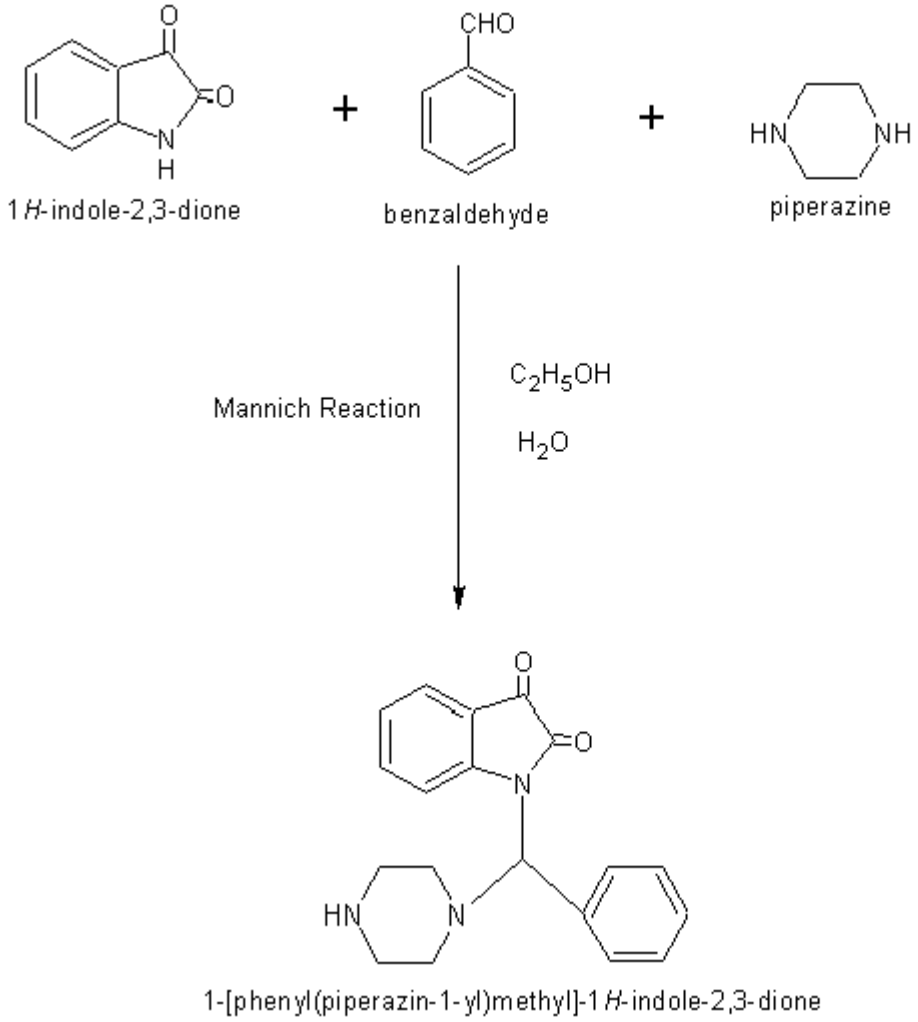
- Isatin -2mmol
- Piperazine -2mmol
- Benzaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and benzaldehyde (3mmol) and add Piperazine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene. Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S2

Synthesis of 1-(phenyl(piperazine-1-yl)-H-indole-2,3 dione



COMPOUND S3

Synthesis of 1-[(4 chlorophenyl)piperazine-1-yl methyl]-1H-indole-2, 3 –dione

Chemicals required:

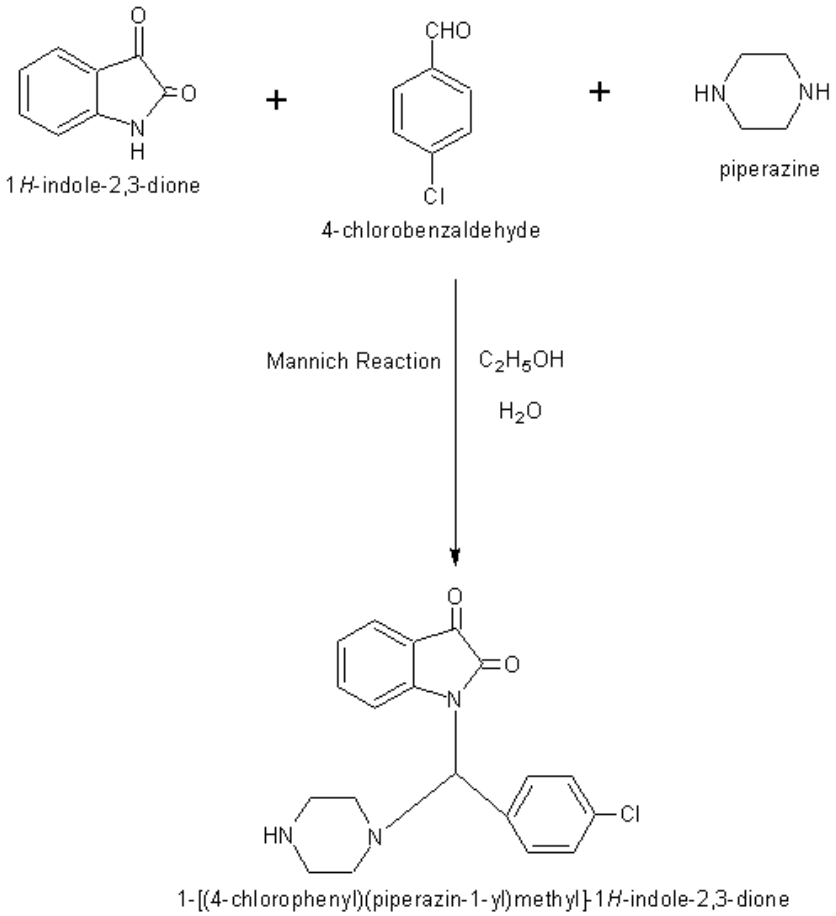
- Isatin -2mmol
- Piperazine -2mmol
- P-chloro Benzaldehyde-3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and 4-chloro Benzaldehyde (3mmol) and add piperazine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene:Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S3

Synthesis of 1-[(4 chlorophenyl)piperazine-1-yl methyl]-1H-indole-2, 3 –dione



COMPOUND S4

Synthesis of 1-[(4 –dimethyl amino) (Piperazine-1-yl methyl]-1H-indole-2, 3 –dione

Chemicals required:

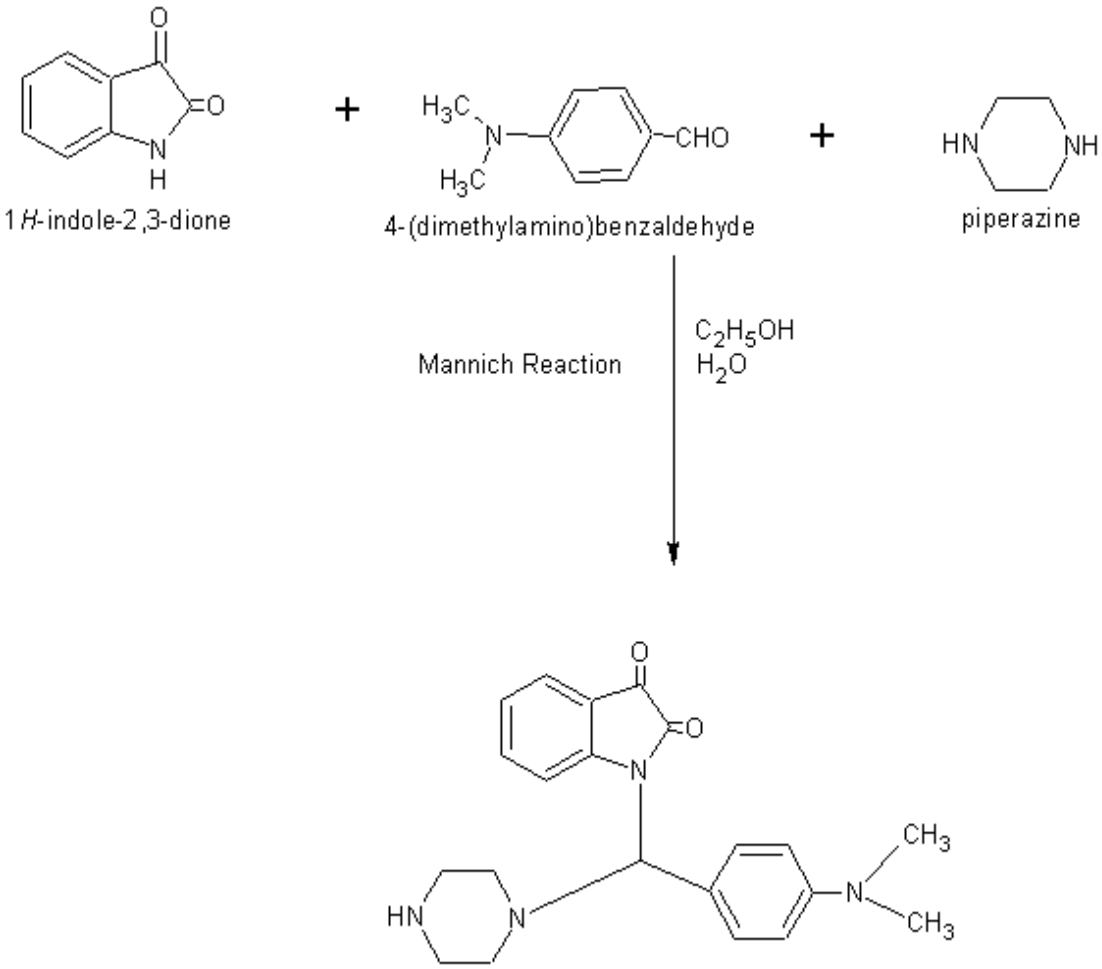
- Isatin -2mmol
- Piperazine -2mmol
- P- dimethyl amino benzaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and p-dimethyl amino benzaldehyde (3mmol) and add piperazine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene: Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S4

Synthesis of 1-[(4 –dimethyl amino) (Piperazine-1-yl methyl]-1H-indole-2, 3 –dione



1-[(4 –dimethyl amino) (Piperazine-1-yl methyl]-1H-indole-2, 3 –dione

COMPOUND S5

Synthesis of 1-[(4 methoxy phenyl)(Piperazine-1-yl methyl]-1H-indole-2, 3 –dione

Chemicals required:

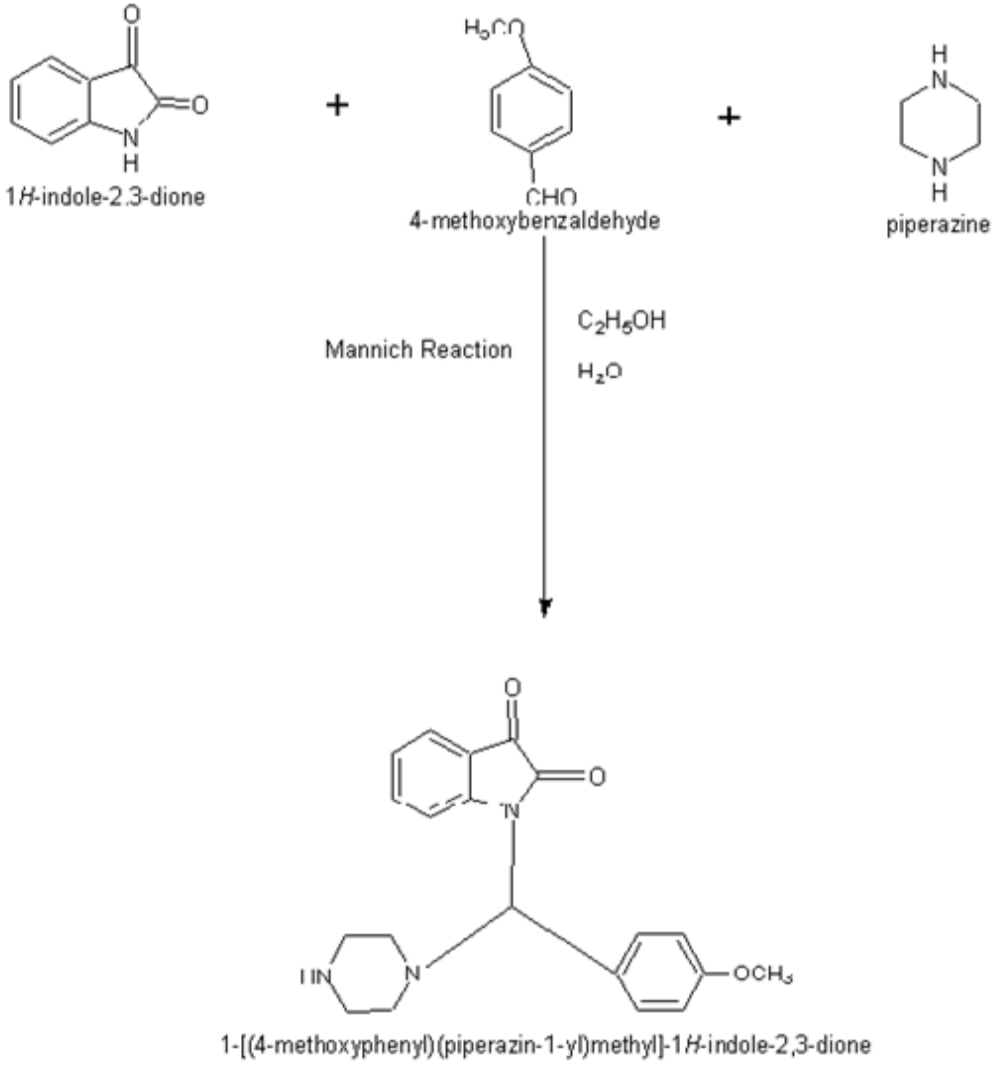
- Isatin -2mmol
- Piperazine -2mmol
- Anisaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and anisaldehyde (3mmol) and add piperazine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene. Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S5

Synthesis of 1-[(4 methoxy phenyl)(Piperazine-1-yl methyl]-1H-indole-2, 3 –dione



COMPOUND S6

Synthesis of 1-[diphenyl amine- yl- methyl]-1H-indole-2, 3 –dione

Chemicals required:

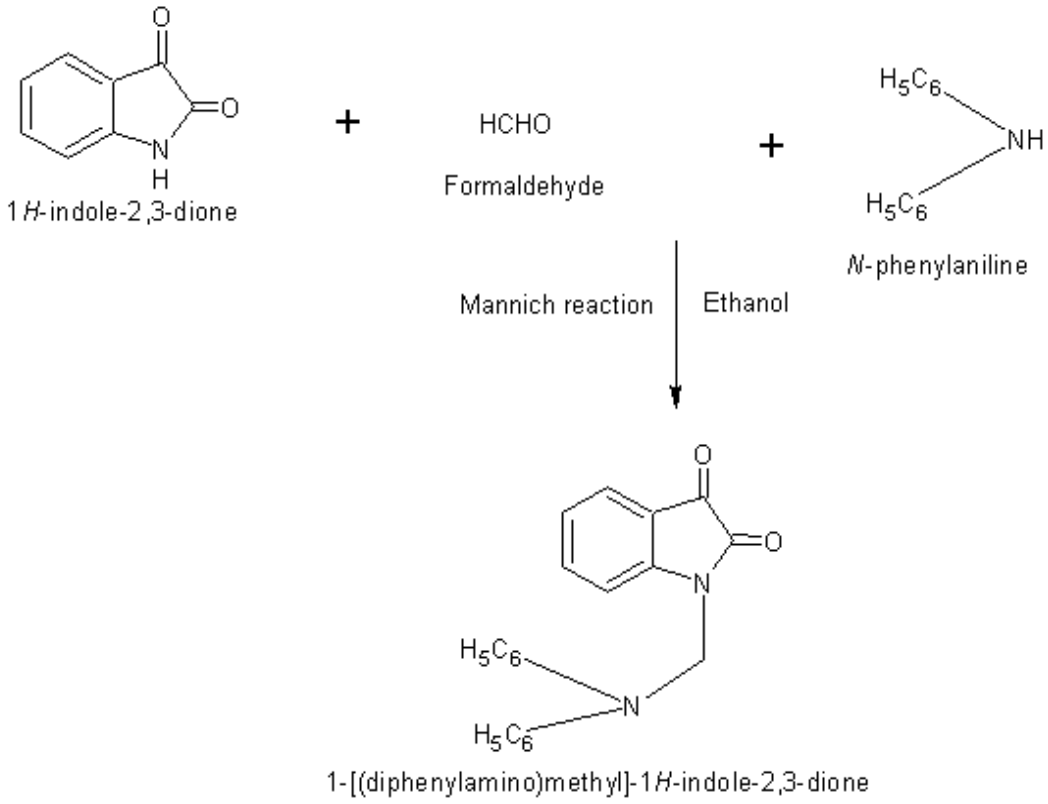
- Isatin -2mmol
- diphenylamine-2mmol
- Formaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and formaldehyde 37% (3mmol) and add diphenylamine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene: Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S6

Synthesis of 1-[diphenyl amine- yl- methyl]-1H-indole-2, 3 –dione



COMPOUND S7

Synthesis of 1-[Diphenylamine,phenyl-1-yl methyl]-1H-indole-2, 3 –dione

Chemicals required:

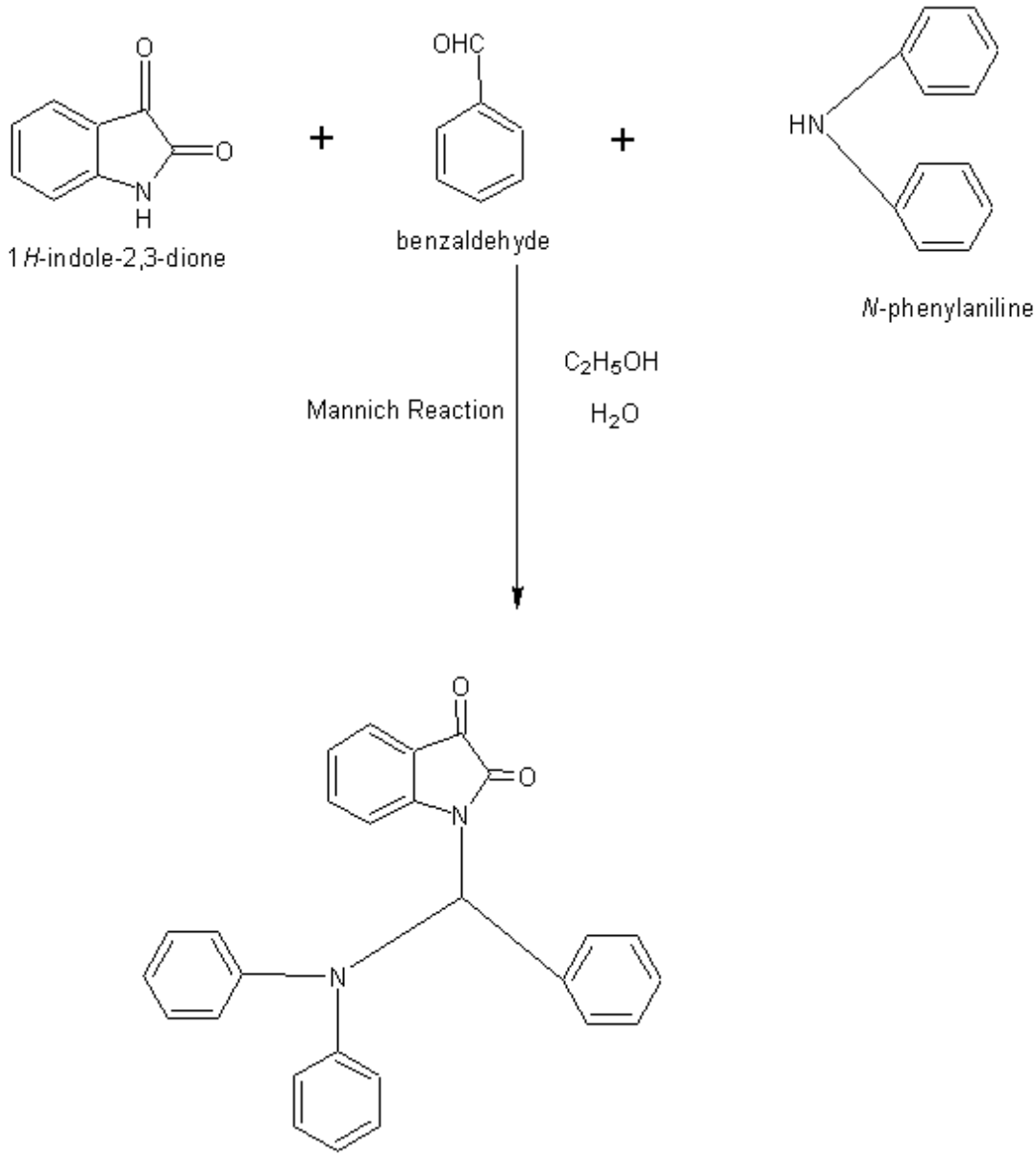
- Isatin -2mmol
- Diphenylamine-2mmol
- Benzaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and Benzaldehyde (3mmol) and add Dyphenylamine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene. Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S7

Synthesis of 1-[Diphenylamine,phenyl-1-yl methyl]-1H-indole-2, 3 –dione



1-[Diphenylamine,phenyl-1-yl methyl]-1H-indole-2, 3 –dione

COMPOUND S8

Synthesis of 1-[1H-benzimidazole-1-yl methyl]-1H-indole-2, 3 –dione

Chemicals required:

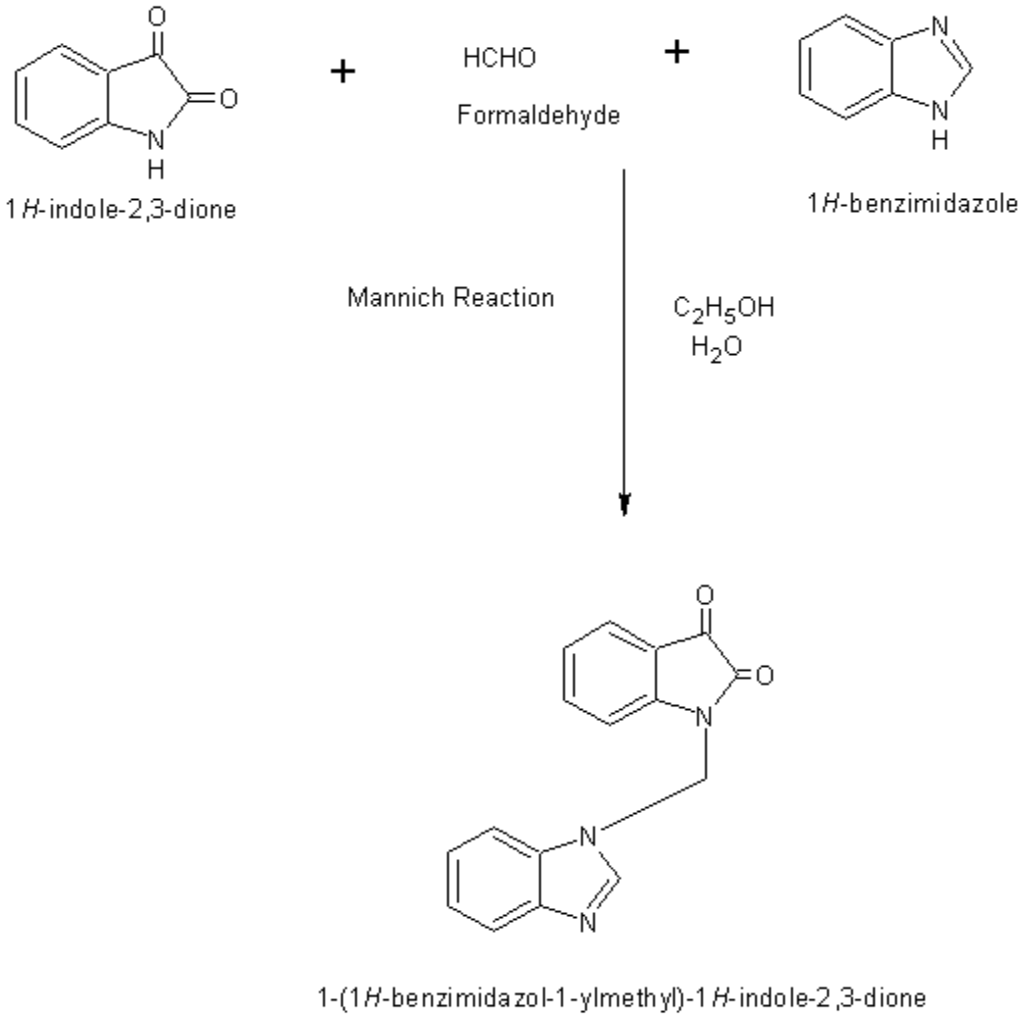
- Isatin -2mmol
- Benzimidazole-2mmol
- Formaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and formaldehyde 37% (3mmol) and add Benzimidazole (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene. Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S8

Synthesis of 1-[1H-benzimidazole-1-yl methyl]-1H-indole-2, 3 –dione



COMPOUND S9

Synthesis of 1-(pyrrolidine-1-yl methyl) -1H –indole-2,3 dione

Chemicals required:

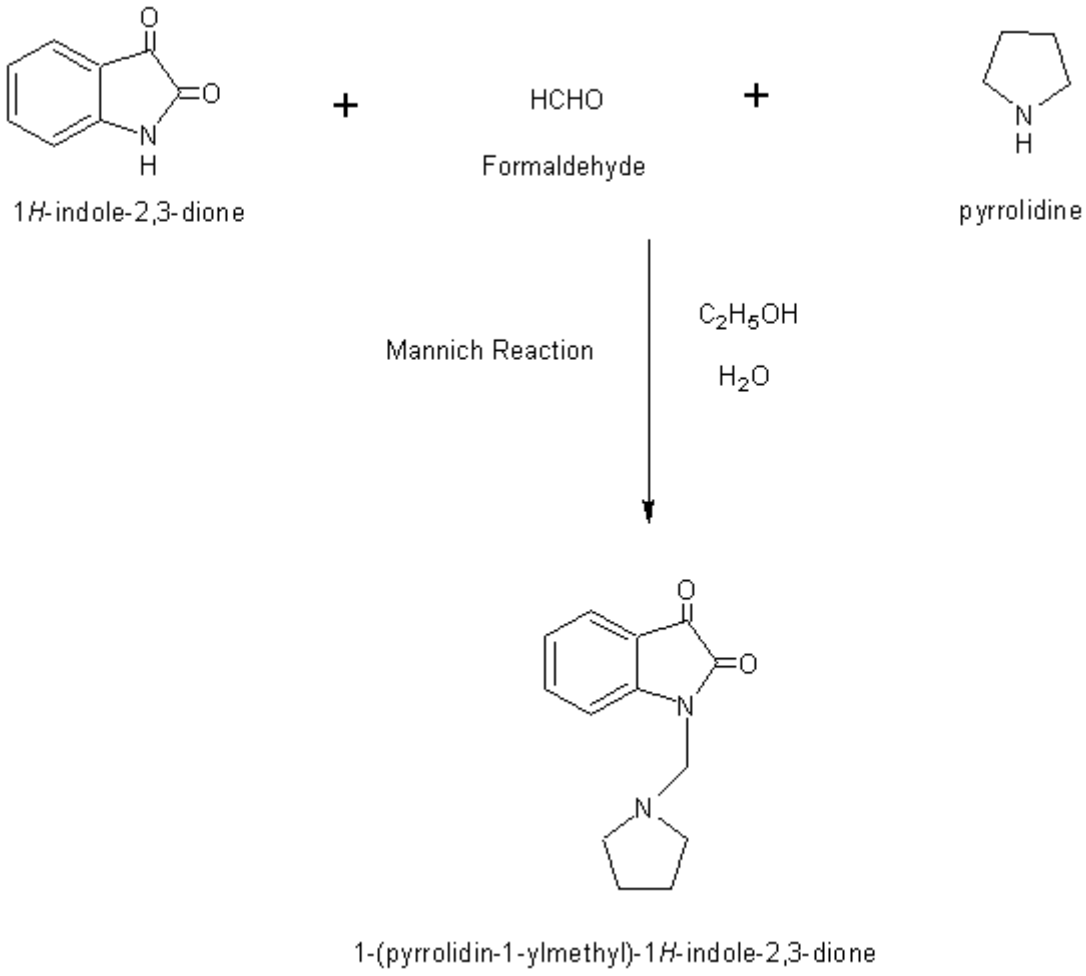
- Isatin -2mmol
- Pyrrolidine -2mmol
- Formaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Isatin (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and formaldehyde 37% (3mmol) and add pyrrolidine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene. Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

COMPOUND S9

Synthesis of1-(pyrrolidine-1-yl methyl) -1H –indole-2,3 dione



COMPOUND S10

Synthesis of 3-(Piperazine-1-yl methyl)-1H-indole

Chemicals required:

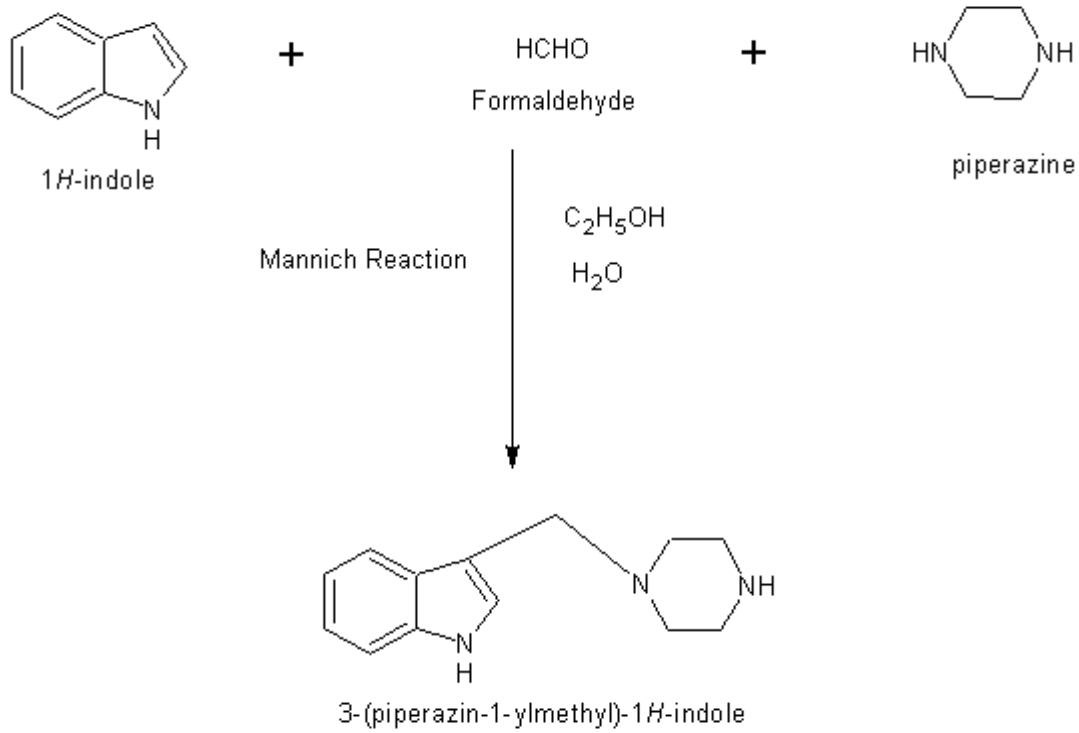
- Indole -2mmol
- Piperazine -2mmol
- Formaldehyde -3mmol
- Ethanol -10ml
- Water -10ml

Procedure:

Indole (2mmol) was dissolved in 20ml of ethanol water (1:1) solution and formaldehyde 37% (3mmol) and add piperazine (2mmol). The mixture was stirred at room temperature and the reaction was controlled by TLC in benzene. Methanol (9:1) at the end of reaction the precipitate was filtered dried and crystallized by using an appropriate solvent.

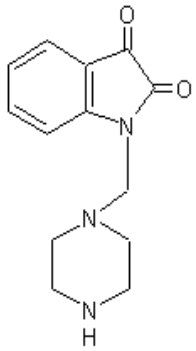
COMPOUND S10

Synthesis of 3-(Piperazine-1-yl methyl)-1H-indole



MOLECULAR DESIGN

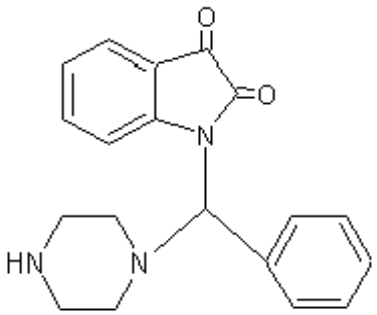
COMPOUND S1



1-(piperazin-1-ylmethyl)-1*H*-indole-2,3-dione

Molecular Formula	= $C_{13}H_{15}N_3O_2$
Formula Weight	= 245.2771
Composition	= C(63.66%) H(6.16%) N(17.13%) O(13.05%)
Molar Refractivity	= 65.69 ± 0.3 cm^3
Molar Volume	= 189.7 ± 3.0 cm^3
Parachor	= 514.5 ± 6.0 cm^3
Index of Refraction	= 1.608 ± 0.02
Surface Tension	= 54.0 ± 3.0 dyne/cm
Density	= 1.292 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 26.04 ± 0.5 $10^{-24} cm^3$
Monoisotopic Mass	= 245.116427 Da
Nominal Mass	= 245 Da
Average Mass	= 245.2771 Da

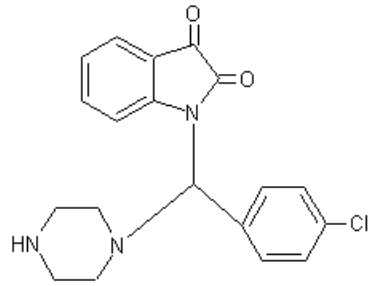
COMPOUND S2



1-[phenyl(piperazin-1-yl)methyl]-1*H*-indole-2,3-dione

Molecular Formula	= $C_{19}H_{19}N_3O_2$
Formula Weight	= 321.37306
Composition	= C(71.01%) H(5.96%) N(13.08%) O(9.96%)
Molar Refractivity	= 90.17 ± 0.3 cm^3
Molar Volume	= 247.7 ± 3.0 cm^3
Parachor	= 686.3 ± 6.0 cm^3
Index of Refraction	= 1.648 ± 0.02
Surface Tension	= 58.9 ± 3.0 dyne/cm
Density	= 1.297 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 35.74 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 321.147727 Da
Nominal Mass	= 321 Da
Average Mass	= 321.3731 Da
M+	= 321.147178 Da
M-	= 321.148275 Da
[M+H]+	= 322.155003 Da
[M+H]-	= 322.1561 Da
[M-H]+	= 320.139353 Da
[M-H]-	= 320.14045 Da

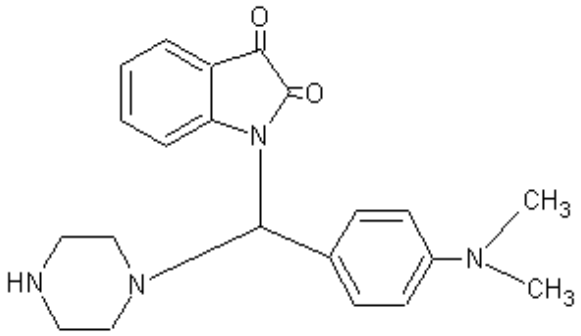
COMPOUND S3



1-[(4-chlorophenyl)(piperazin-1-yl)methyl]-1*H*-indole-2,3-dione

Molecular Formula	= $C_{19}H_{18}ClN_3O_2$
Formula Weight	= 355.81812
Composition	= C(64.13%) H(5.10%) Cl(9.96%) N(11.81%) O(8.99%)
Molar Refractivity	= 95.06 ± 0.3 cm^3
Molar Volume	= 259.6 ± 3.0 cm^3
Parachor	= 723.5 ± 6.0 cm^3
Index of Refraction	= 1.653 ± 0.02
Surface Tension	= 60.2 ± 3.0 dyne/cm
Density	= 1.370 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 37.68 ± 0.5 $10^{-24} cm^3$
Monoisotopic Mass	= 355.108755 Da
Nominal Mass	= 355 Da
Average Mass	= 355.8181 Da

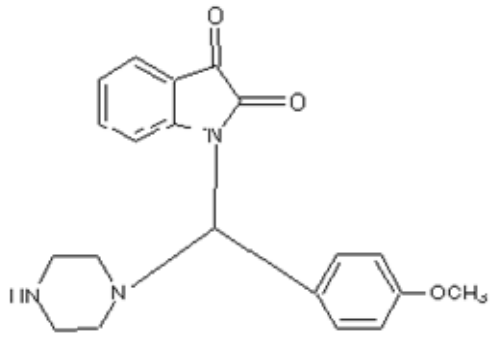
COMPOUND S4



1-[(4 –dimethyl amino) (Piperazine-1-yl methyl]-1H-indole-2, 3 –dione

Molecular Formula	= $C_{21}H_{24}N_4O_2$
Formula Weight	= 364.44086
Composition	= C(69.21%) H(6.64%) N(15.37%) O(8.78%)
Molar Refractivity	= 104.48 ± 0.3 cm^3
Molar Volume	= 285.7 ± 3.0 cm^3
Parachor	= 791.0 ± 6.0 cm^3
Index of Refraction	= 1.652 ± 0.02
Surface Tension	= 58.7 ± 3.0 dyne/cm
Density	= 1.275 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 41.42 ± 0.5 $10^{-24} cm^3$
Monoisotopic Mass	= 364.189926 Da
Nominal Mass	= 364 Da
Average Mass	= 364.4409 Da

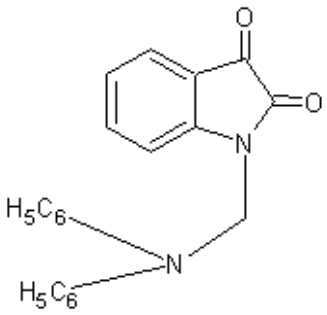
COMPOUND S5



1-[(4-methoxyphenyl)(piperazin-1-yl)methyl]-1*H*-indole-2,3-dione

Molecular Formula	= $C_{20}H_{21}N_3O_3$
Formula Weight	= 351.39904
Composition	= C(68.36%) H(6.02%) N(11.96%) O(13.66%)
Molar Refractivity	= 96.84 ± 0.3 cm^3
Molar Volume	= 271.7 ± 3.0 cm^3
Parachor	= 745.0 ± 6.0 cm^3
Index of Refraction	= 1.631 ± 0.02
Surface Tension	= 56.4 ± 3.0 dyne/cm
Density	= 1.293 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 38.39 ± 0.5 10^{-24} cm^3
Monoisotopic Mass	= 351.158292 Da
Nominal Mass	= 351 Da
Average Mass	= 351.399 Da

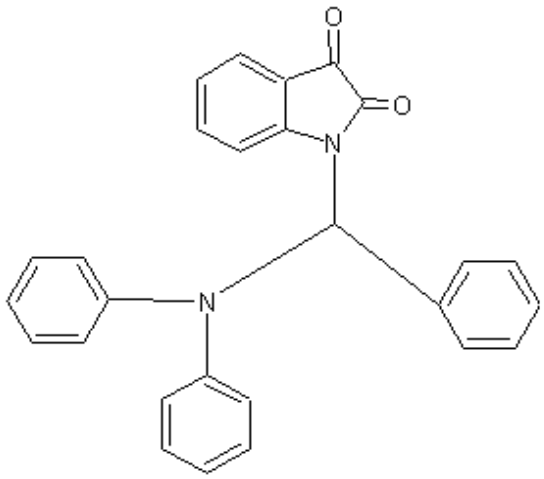
COMPOUND S6



1-[(diphenylamino)methyl]-1*H*-indole-2,3-dione

Molecular Formula	= $C_{21}H_{16}N_2O_2$
Formula Weight	= 328.36394
Composition	= C(76.81%) H(4.91%) N(8.53%) O(9.74%)
Molar Refractivity	= 95.98 ± 0.3 cm^3
Molar Volume	= 252.6 ± 3.0 cm^3
Parachor	= 703.2 ± 6.0 cm^3
Index of Refraction	= 1.684 ± 0.02
Surface Tension	= 60.0 ± 3.0 dyne/cm
Density	= 1.299 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 38.05 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 328.121178 Da
Nominal Mass	= 328 Da
Average Mass	= 328.3639 Da

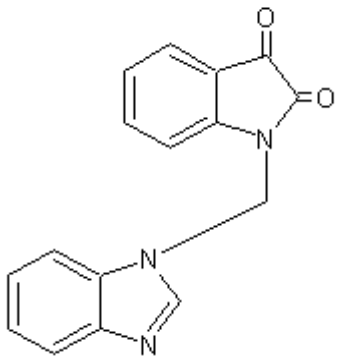
COMPOUND S7



1-[Diphenylamine,phenyl-1-yl methyl]-1H-indole-2, 3 –dione

Molecular Formula	= $C_{21}H_{20}N_2O_2$
Formula Weight	= 404.4599
Composition	= C(80.18%) H(4.98%) N(6.93%) O(7.91%)
Molar Refractivity	= 120.46 ± 0.3 cm^3
Molar Volume	= 310.5 ± 3.0 cm^3
Parachor	= 875.0 ± 6.0 cm^3
Index of Refraction	= 1.703 ± 0.02
Surface Tension	= 62.9 ± 3.0 dyne/cm
Density	= 1.302 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 47.75 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 404.152478 Da
Nominal Mass	= 404 Da
Average Mass	= 404.4599 Da

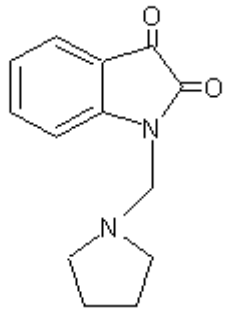
COMPOUND S8



1-(1*H*-benzimidazol-1-ylmethyl)-1*H*-indole-2,3-dione

Molecular Formula	= $C_{16}H_{11}N_3O_2$
Formula Weight	= 277.27744
Composition	= C(69.31%) H(4.00%) N(15.15%) O(11.54%)
Molar Refractivity	= 78.75 ± 0.5 cm^3
Molar Volume	= 196.9 ± 7.0 cm^3
Parachor	= 552.2 ± 8.0 cm^3
Index of Refraction	= 1.731 ± 0.05
Surface Tension	= 61.7 ± 7.0 dyne/cm
Density	= 1.40 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 31.22 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 277.085127 Da
Nominal Mass	= 277 Da
Average Mass	= 277.2774 Da

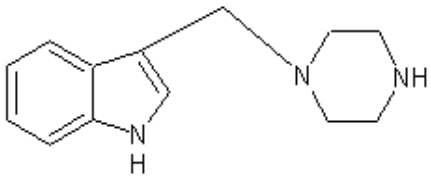
COMPOUND S9



1-(pyrrolidin-1-ylmethyl)-1*H*-indole-2,3-dione

Molecular Formula	= $C_{13}H_{14}N_2O_2$
Formula Weight	= 230.26246
Composition	= C(67.81%) H(6.13%) N(12.17%) O(13.90%)
Molar Refractivity	= 62.34 ± 0.3 cm^3
Molar Volume	= 175.9 ± 3.0 cm^3
Parachor	= 486.3 ± 6.0 cm^3
Index of Refraction	= 1.626 ± 0.02
Surface Tension	= 58.3 ± 3.0 dyne/cm
Density	= 1.308 ± 0.06 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 24.71 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 230.105528 Da
Nominal Mass	= 230 Da
Average Mass	= 230.2625 Da

COMPOUND S10

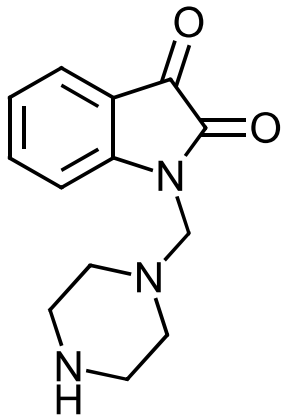


3-(piperazin-1-ylmethyl)-1*H*-indole

Molecular Formula	= $C_{12}H_{15}N_3$
Formula Weight	= 201.2676
Composition	= C(71.61%) H(7.51%) N(20.88%)
Molar Refractivity	= 61.16 ± 0.5 cm^3
Molar Volume	= 166.5 ± 7.0 cm^3
Parachor	= 441.2 ± 8.0 cm^3
Index of Refraction	= 1.655 ± 0.05
Surface Tension	= 49.3 ± 7.0 dyne/cm
Density	= 1.20 ± 0.1 g/cm^3
Dielectric Constant	= Not available
Polarizability	= 24.24 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	= 201.126597 Da
Nominal Mass	= 201 Da
Average Mass	= 201.2676 Da

1(piperazine-1-ylmethyl)-1H –indole-2,3 dione

S1



Molecular Formula = $C_{13}H_{15}N_3O_2$

Molecular Mass = 245.2771 u

Hydrogen Bond Donor Count = 1

Hydrogen Bond Acceptor Count = 4

T_f = 408.6700 K

T_b = 582.6500 K

CMR = 69.3390 cm^3/mol

AMR = 70.3717 cm^3/mol

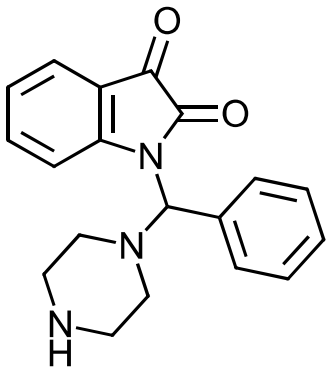
XlogP v2.0 = 1.9830

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-(phenyl(piperazine-1-yl)-H-indole-2,3 dione

S2



Molecular Formula = $C_{19}H_{19}N_3O_2$

Molecular Mass = 321.3730 u

Hydrogen Bond Acceptor Count = 4

Hydrogen Bond Donor Count = 1

T_b = 730.4500 K

T_f = 464.8300 K

XlogP v2.0 = 3.5220

CMR = 94.4510 cm^3/mol

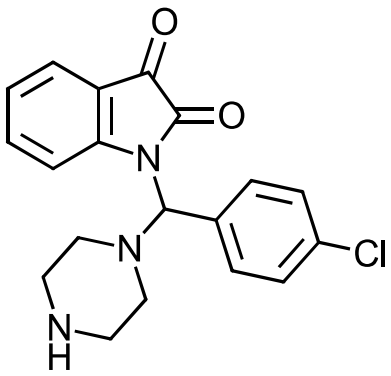
AMR = 94.8367 cm^3/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-[(4 chlorophenyl)piperazine-1-yl methyl]-1H-indole-2, 3 –dione

S3



Molecular Formula = $C_{19}H_{18}ClN_3O_2$

Molecular Mass = 355.8181 u

Hydrogen Bond Acceptor Count = 4

Hydrogen Bond Donor Count = 1

T_b = 767.7599 K

T_f = 480.7900 K

XlogP v2.0 = 4.1440

CMR = 99.3650 cm^3/mol

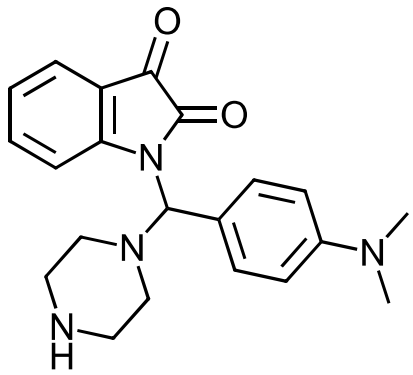
AMR = 99.8467 cm^3/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-[(4 –dimethyl amino) (Piperazine-1-yl methyl]-1H-indole-2, 3 –dione

S4



Molecular Formula = $C_{21}H_{24}N_4O_2$

Molecular Mass = 364.4409 u

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 1

T_b = 788.5300 K

T_f = 505.8800 K

XlogP v2.0 = 4.1530

CMR = 107.4140 cm^3/mol

AMR = 109.1637 cm^3/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

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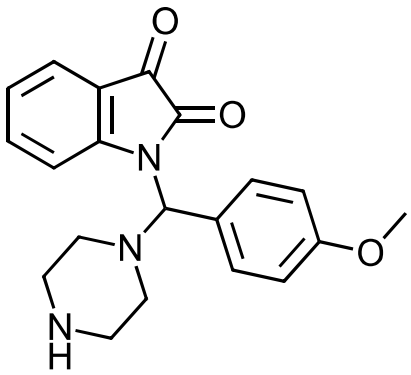
1-[(4 methoxy phenyl)(Piperazine-1-yl methyl]-1H-indole-2, 3 –dione

S5



Molecular Formula = $C_{20}H_{21}N_3O_3$

Molecular Mass = 351.3990 u

Hydrogen Bond Acceptor Count = 5

Hydrogen Bond Donor Count = 1

T_b = 775.6300 K

T_f = 484.3701 K

XlogP v2.0 = 3.8590

CMR = 100.6200 cm^3/mol

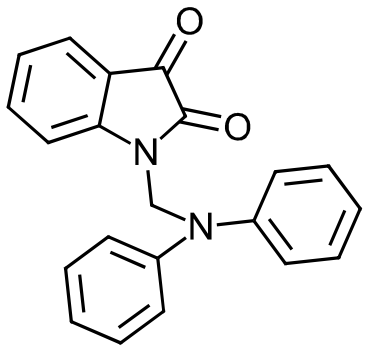
AMR = 101.3887 cm^3/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-[diphenyl amine- yl- methyl]-1H-indole-2, 3 –dione

S6



Molecular Formula = $C_{21}H_{16}N_2O_2$

Molecular Mass = 328.3639 u

Hydrogen Bond Acceptor Count = 3

Hydrogen Bond Donor Count = 0

T_b = 738.8400 K

T_f = 420.5100 K

XlogP v2.0 = 4.3830

CMR = 99.0980 cm^3/mol

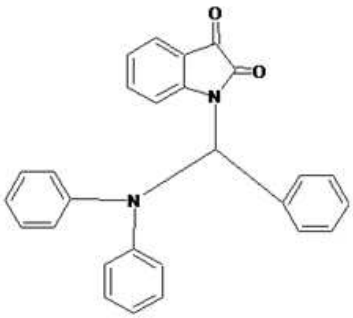
AMR = 100.9520 cm^3/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-[Diphenylamine,phenyl-1-yl methyl]-1H-indole-2, 3 –dione

S7



Molecular Formula = C27H20N2O2

Molecular Mass = 404.4599

Hydrogen Bond Acceptor Count = 4

Hydrogen Bond Donor Count = 1

T_b = 730.4500 K

T_f = 464.8300 K

XlogP v2.0 = 3.5220

CMR = 94.4510 cm³/mol

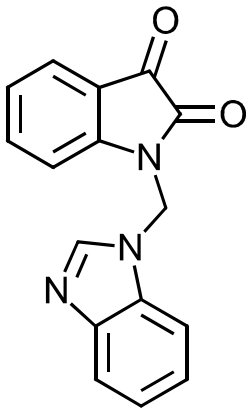
AMR = 94.8367 cm³/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-[1H-benzimidazole-1-yl methyl]-1H-indole-2, 3 –dione

S8



Molecular Formula = $C_{16}H_{11}N_3O_2$

Molecular Mass = 277.2774 u

Hydrogen Bond Acceptor Count = 2

Hydrogen Bond Donor Count = 0

T_b = 688.6400 K

T_f = 376.8600 K

XlogP v2.0 = 1.2250

CMR = 79.3630 cm³/mol

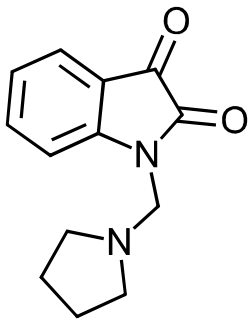
AMR = 80.7950 cm³/mol

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

1-(pyrrolidine-1-yl methyl) -1H –indole-2,3 dione

S9



Molecular Formula = $C_{13}H_{14}N_2O_2$

Molecular Mass = 230.2625 u

Hydrogen Bond Acceptor Count = 3

Hydrogen Bond Donor Count = 0

T_b = 532.4800 K

T_f = 356.0100 K

CMR = 65.6520 cm^3/mol

AMR = 66.7360 cm^3/mol

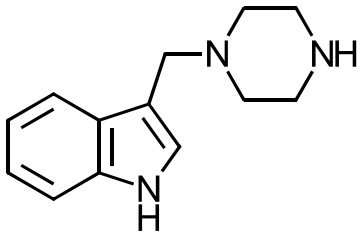
XlogP v2.0 = 2.4250

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

3-(Piperazine-1-yl methyl)-1H-indole

S10



Molecular Formula = $C_{13}H_{17}N_3$

Molecular Mass = 215.2942 u

Hydrogen Bond Donor Count = 2

Hydrogen Bond Acceptor Count = 2

T_f = 410.0800 K

T_b = 621.9000 K

CMR = 66.8510 cm³/mol

AMR = 66.7104 cm³/mol

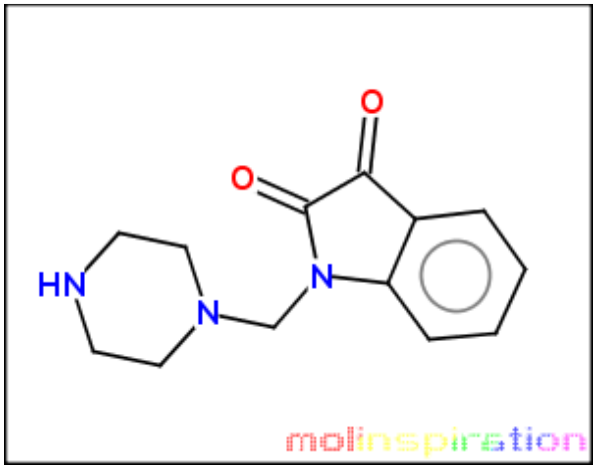
XlogP v2.0 = 3.5860

Bioavailability Score = 0.5500

Lipinski's Rule of 5 Violations Count = 0

MOLINSPIRATION

S1



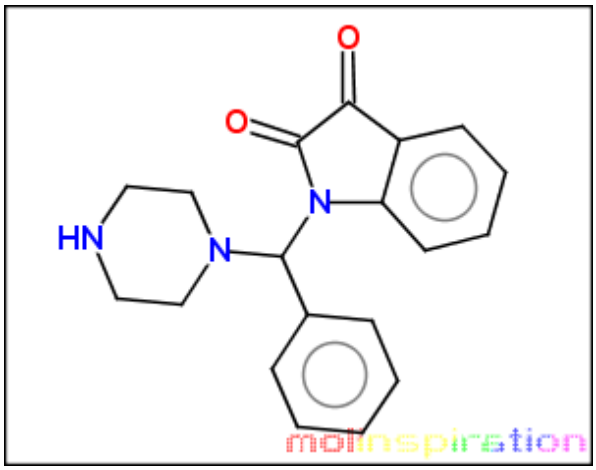
Molinspiration property engine v2013.09

miLogP	0.211
TPSA	54.341
natoms	18.0
MW	245.282
nON	5
nOHNH	1
nviolations	0
nrotb	2
volume	222.30

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.40
Ion channel modulator	-0.66
Kinase inhibitor	-0.34
Nuclear receptor ligand	-1.23
Protease inhibitor	-0.66
Enzyme inhibitor	-0.05

S2



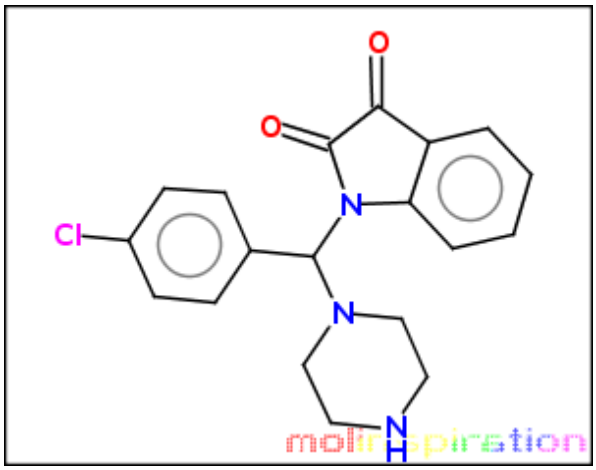
Molinspiration property engine v2013.09

miLogP	1.793
TPSA	54.341
natoms	24.0
MW	321.38
nON	5
nOHNH	1
nviolations	0
nrotb	3
volume	293.741

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.11
Ion channel modulator	-0.39
Kinase inhibitor	-0.16
Nuclear receptor ligand	-0.55
Protease inhibitor	-0.08
Enzymeinhibitor	-0.14

S3



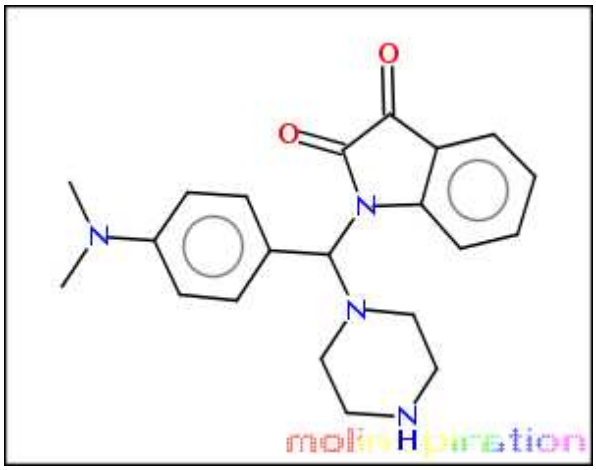
Molinspiration property engine v2013.09

miLogP	2.471
TPSA	54.341
natoms	25.0
MW	355.825
nON	5
nOHNH	1
nviolations	0
nrotb	3
volume	307.276

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.11
Ion channel modulator	-0.38
Kinase inhibitor	-0.18
Nuclear receptor ligand	-0.55
Protease inhibitor	-0.12
Enzyme inhibitor	-0.17

S4



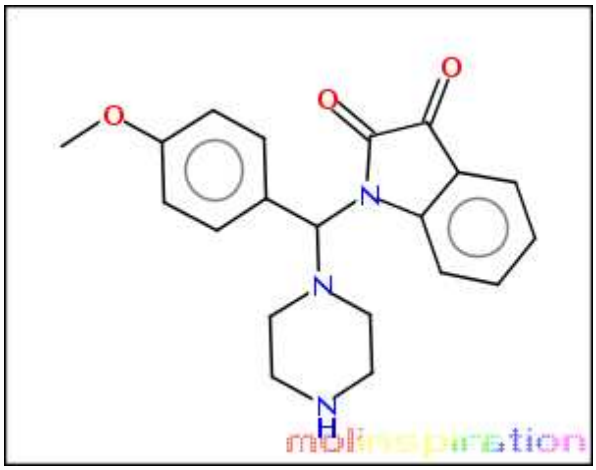
Molinspiration property engine v2013.09

miLogP	1.895
TPSA	57.579
natoms	27.0
MW	364.449
nON	6
nOHNH	1
nviolations	0
nrotb	4
volume	339.647

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.10
Ion channel modulator	-0.37
Kinase inhibitor	-0.12
Nuclear receptor ligand	-0.48
Protease inhibitor	-0.10
Enzymeinhibitor	-0.15

S5



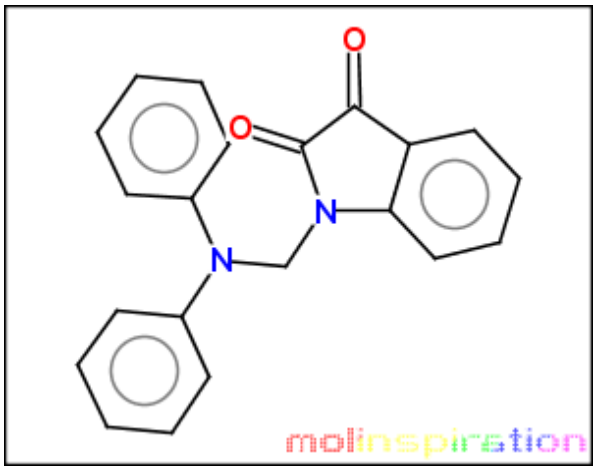
Molinspiration property engine v2013.09

miLogP	1.85
TPSA	63.575
natoms	26.0
MW	351.406
nON	6
nOHNH	1
nviolations	0
nrotb	4
volume	319.286

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.15
Ion channel modulator	-0.43
Kinase inhibitor	-0.19
Nuclear receptor ligand	-0.52
Protease inhibitor	-0.13
Enzymeinhibitor	-0.18

S6



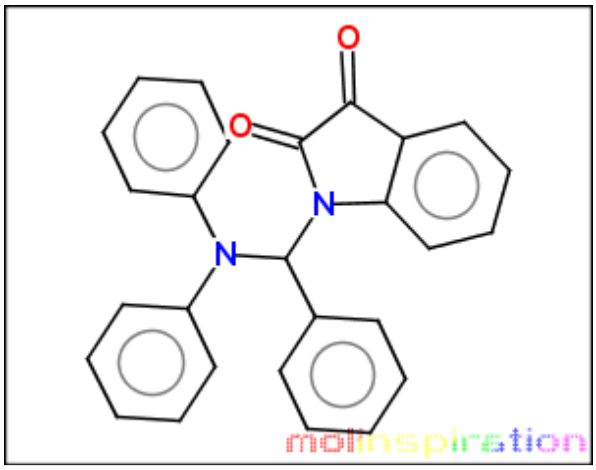
Molinspiration property engine v2013.09

miLogP	4.31
TPSA	42.314
natoms	25.0
MW	328.371
nON	4
nOHNH	0
nviolations	0
nrotb	4
volume	296.356

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.11
Ion channel modulator	-0.35
Kinase inhibitor	-0.07
Nuclear receptor ligand	-0.42
Protease inhibitor	-0.19
Enzymeinhibitor	0.03

S7



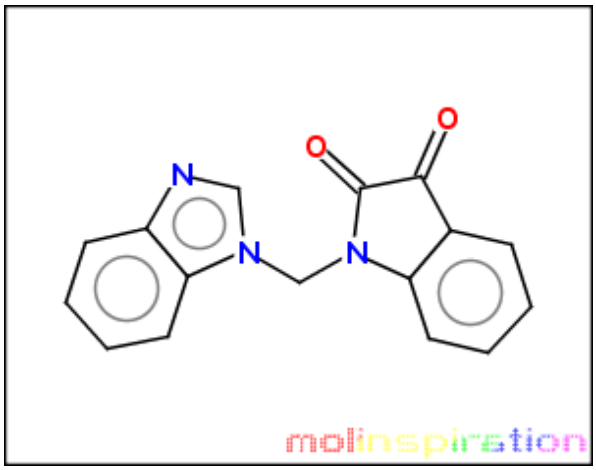
Molinspiration property engine v2013.09

miLogP	5.891
TPSA	42.314
natoms	31.0
MW	404.469
nON	4
nOHNH	0
nviolations	1
nrotb	5
volume	367.79

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.14
Ion channel modulator	-0.35
Kinase inhibitor	-0.14
Nuclear receptor ligand	-0.34
Protease inhibitor	-0.15
Enzymeinhibitor	-0.11

S8



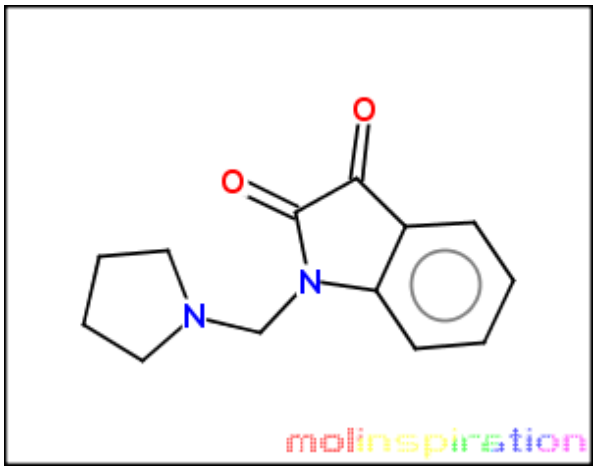
Molinspiration property engine v2013.09

miLogP	2.07
TPSA	56.902
natoms	21.0
MW	277.283
nON	5
nOHNH	0
nviolations	0
nrotb	2
volume	237.366

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.01
Ion channel modulator	-0.30
Kinase inhibitor	0.02
Nuclear receptor ligand	-0.46
Protease inhibitor	-0.12
Enzymeinhibitor	0.08

S9



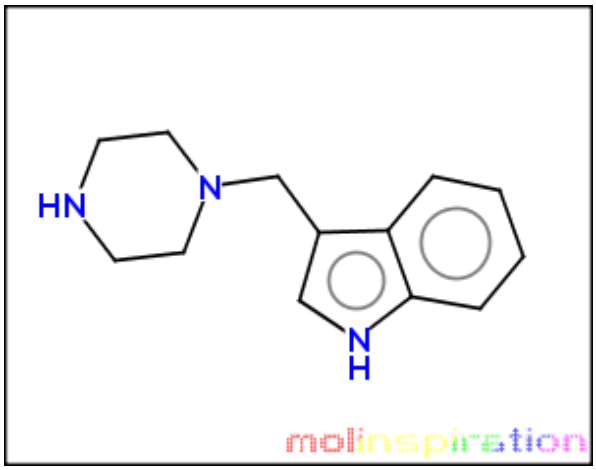
Molinspiration property engine v2013.09

miLogP	1.318
TPSA	42.314
natoms	17.0
MW	230.267
nON	4
nOHNH	0
nviolations	0
nrotb	2
volume	209.904

Molinspiration bioactivity score v2011.06

GPCR ligand	-0.54
Ion channel modulator	-0.69
Kinase inhibitor	-0.52
Nuclear receptor ligand	-1.18
Protease inhibitor	-0.68
Enzymeinhibitor	-0.06

S10



Molinspiration property engine v2013.09

miLogP	1.188
TPSA	31.056
natoms	16.0
MW	215.3
nON	3
nOHNH	2
nviolations	0
nrotb	2
volume	211.374

Molinspiration bioactivity score v2011.06

GPCR ligand	0.20
Ion channel modulator	0.25
Kinase inhibitor	0.11
Nuclear receptor ligand	-0.54
Protease inhibitor	-0.28
Enzymeinhibitor	0.09

ANALYTICAL TECHNICHE

IR SPECTROSCOPY[49]

Infrared spectroscopy or vibrational spectroscopy is concerned with the study of absorption of infrared radiation, which results in vibrational transition. IR spectra is mainly used in structure elucidation to determine the functional group.

Principle:

In any molecule, It is known that atoms or groups of atoms one connected by bonds. These bonds are analogues to springs and not rigid in nature. Because of the continuous motion of the molecule. They maintain some vibrations with some frequency. Characteristic to every portion of the molecule. This is called the natural frequency of vibration.

When energy in the form of infrared radiation is applied and when every bond or portion of a molecule or functional group requires different frequency for absorption. Hence characteristic peak is observed for every functional group or part of the molecule. In other words IR structure is Nothing but a finger print of a molecule. (Main application of IR spectra is identification of functional group and structure elucidation.)

NUCLEAR MAGNETIC RESONANCE[25,26]

The NMR spectroscopy depends upon the fact that most isotopes of the elements posses gyromagnetic properties, meaning thereby that they behave like tiny spinning bar magnets. When a sample containing nuclei exhibiting this immutable gyromagnetism is placed in an appropriate DC Magnetic field and is simultaneously irradiated by weaker rotating radio frequency magnetic field the nuclei can be compelled to

A] Reveal their presence

B] Identify themselves and

C] Describe the nature of their surroundings

All by means of minute radiological which they transmit to a receiver coil coupled closely to the sample.

PRINCIPLE:

NMR involves the interaction between an oscillating magnetic field of electromagnetic radiation and the magnetic energy of the H nucleus when these are placed in an external static magnetic field.

The sample absorbs electromagnetic radiation in radiowave region at different frequency since absorption depends upon the type of protons or certain nuclei contained in the sample consider a spinning top.

It performs a slower waltz like motion in which the spinning axis of the topmoves slowly around the vertical axis. This is precessional motion and the top is said to be precessing around the vertical axis of the earth gravitational field.

The precession arise from the interaction of spin with earth gravity acting vertically downloads. It is known gyroscopic motion.

NMR spectroscopy is the study of spin changes at the nucleus level. When a radio frequency energy is absorbed in the presence of magnetic field. When proton (hydrogen) is studied then its called as proton magnetic resonance (PMR). When other nuclei like C^{13}, ^{19}F, ^{35}Cl etc is studied then it is called as NMR.

Generally in practice the study of hydrogen. Itself is called as NMR spectra. Nuclei with odd mass number only give NMR spectra eg. ^{1}H, ^{13}C, ^{19}F, ^{35}Cl etc. Because they have assymetrical charge distribution.

COMPLICATION IN NMR ANALYSIS

1. Hydrogen bonding
2. Overlapping
3. Solvent effects.
4. Exchange of proton.

LIMITATION IN NMR STUDIES:

- Lack of sensitivity.
- Choice of solvent is restricted.
- Fairly large samples are required minimum sample size is 0.1ml having minimum
- concentration about 1%.
- Limited number of nuclei may be studied.
- In some compounds, two different type of hydrogen atom resonate at some resonancd frequencies .theis result in an overlap of spectra and make such spectra difficult to interpret.
- In most of the cases, only liquid can be studied by NMR spectroscopy.

APPLICATION:

- Structural Diagnoses.
- Complex spectra can be simplified by deuterium labelling.
- Identification of structural isomers.
- Conformational analysis.
- Distinction between cis and trans isomers.
- Detection of hydrogen bonding.
- Detection of electro negative atom.
- Detection of aromaticity.
- Keto-enoltautomerism.
- Elemental analysis
- Detection of Double bond Character.
- Quantitative analysis.

MASS SPECTROSCOPY[26]

Mass or molecules weight of a compound can be found in several ways. One such technique is using mass spectrometer not only for determination of mass. But the technique can be used for structure elucidation, quantitative analysis and even advanced studies could be done by using mass spectrum of a compound.

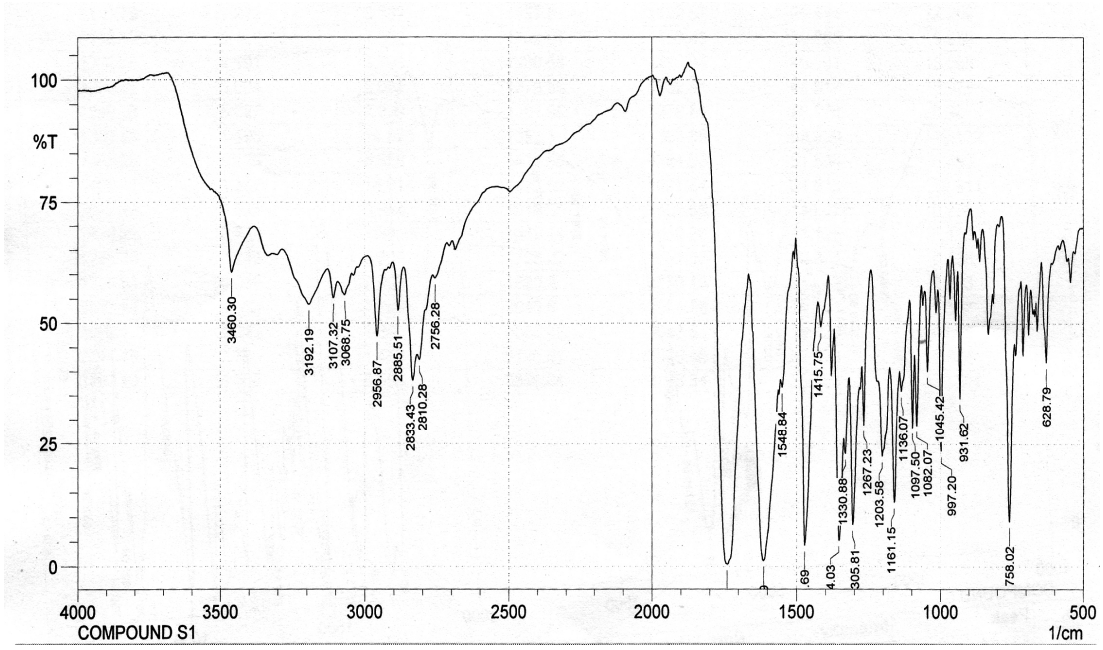
Basic principle:

Mass spectroscopy is the most accurate method for determining the molecular mass of the compounds and its elemental composition. In this technique, the compound under investigation is bombarded with a beam of energetic electrons. The molecules are ionised and dissociate with several fragments, some of which are positive ions each kind of ions have a particular ratio of mass to change ie m/e ratio.

Mass spectra is called as positive ion spectra or ion spectra unlike other kinds of spectroscopy we don't use any electromagnetic radiation [EMR] we use electron bombardment to convert a neutral molecule to positive charged one also there is no ground or excited state like other type of spectroscopy mass spectroscopy is not a true spectroscopic technique because of absorption of electromagnet energy is not involved in any way. The important advantages of mass spectroscopy are its high sensitivity, reproducibility, accuracy and the amount sample required for mass spectral analysis material present in concentration less than 1 ppm can be easily detected by this technique .

IR SPECTROSCOPY

COMPOUND S1



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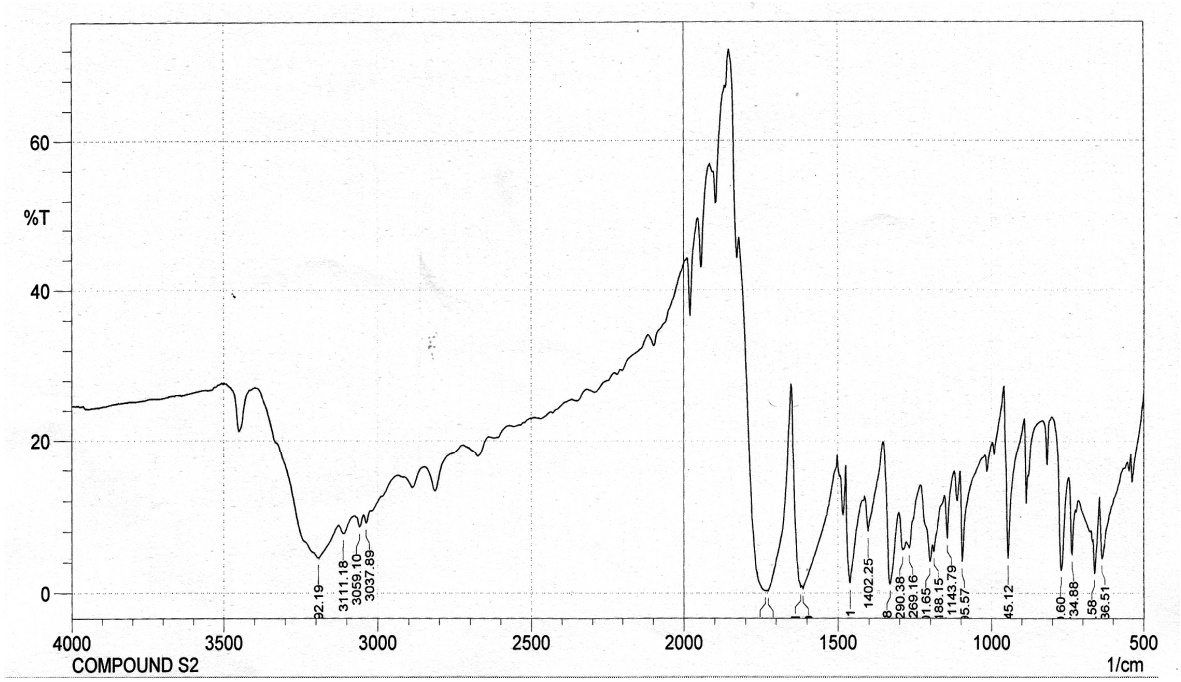

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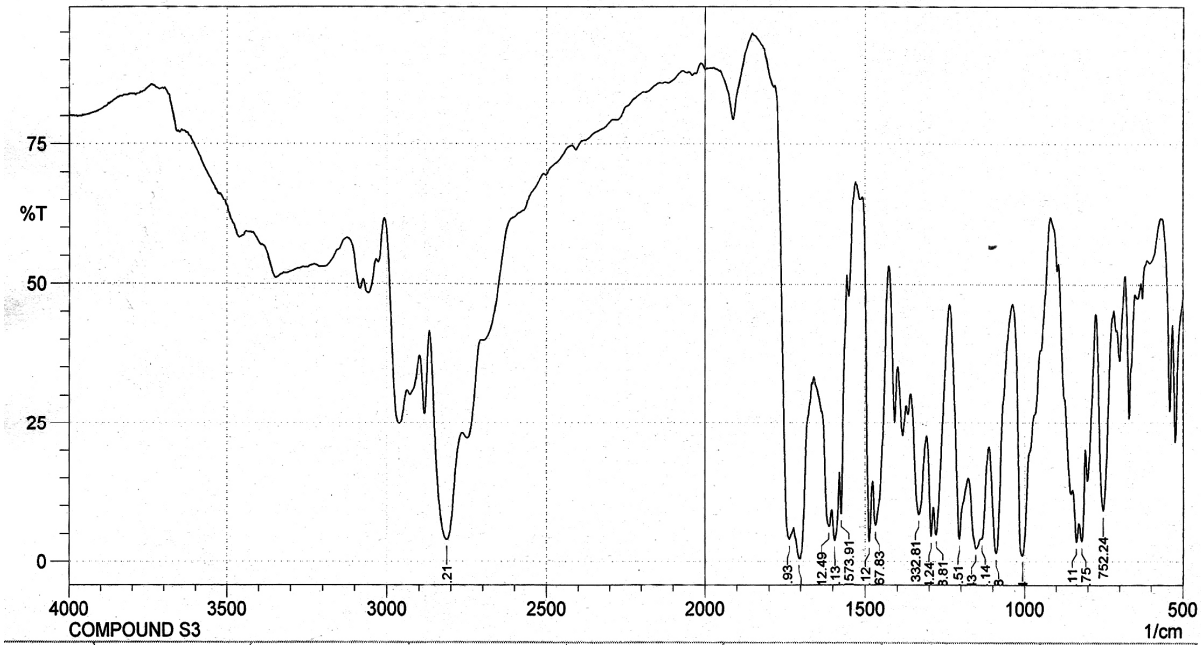

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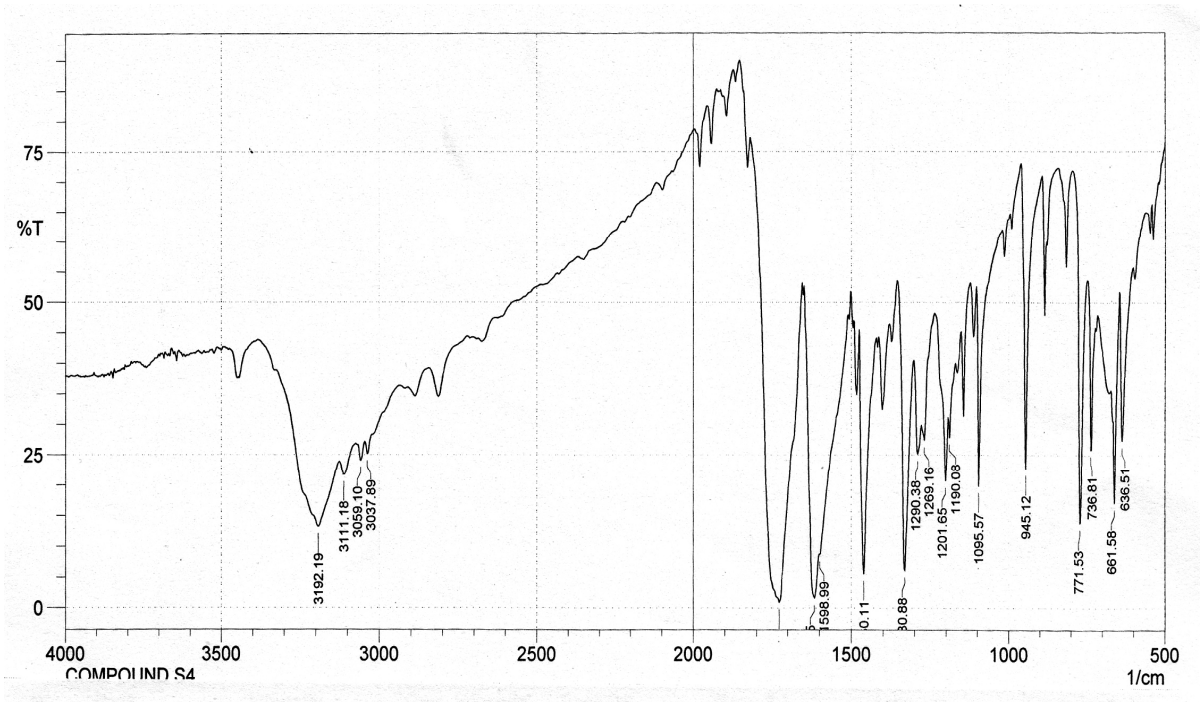

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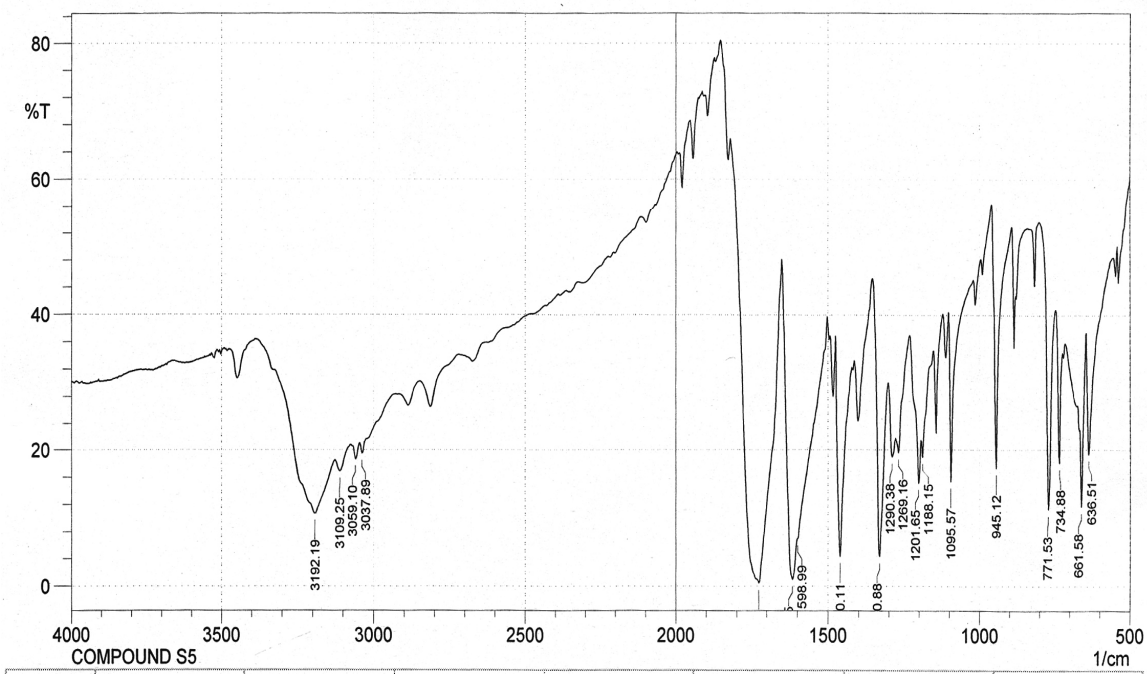

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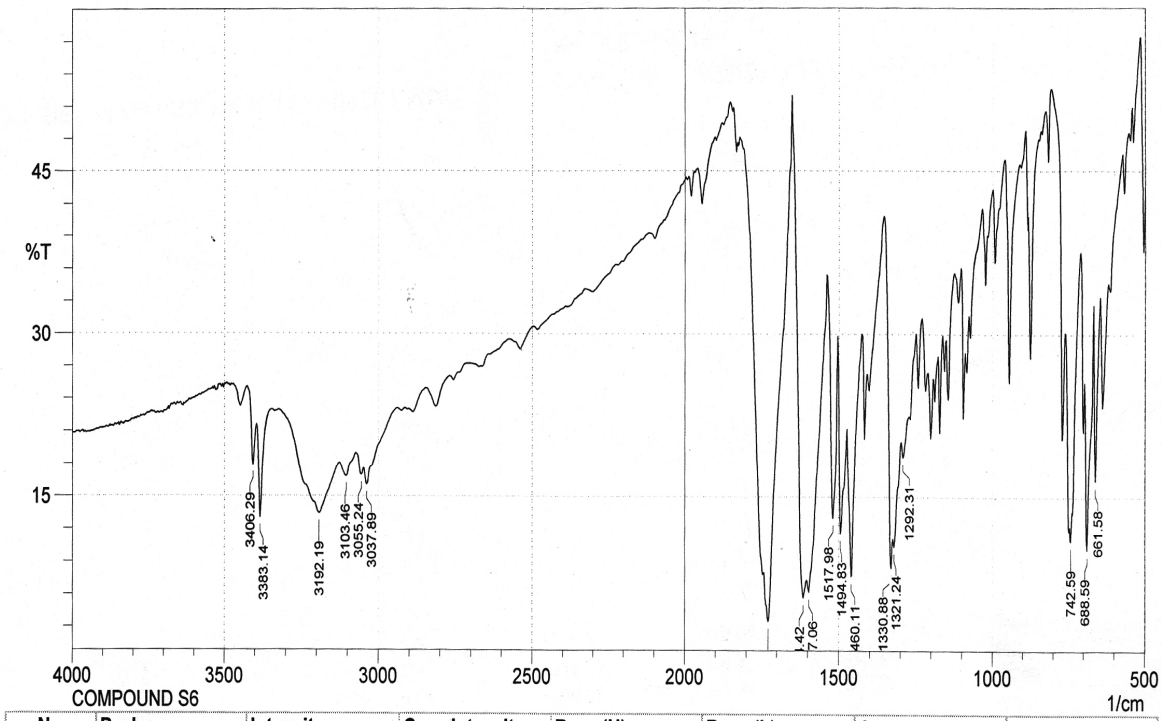

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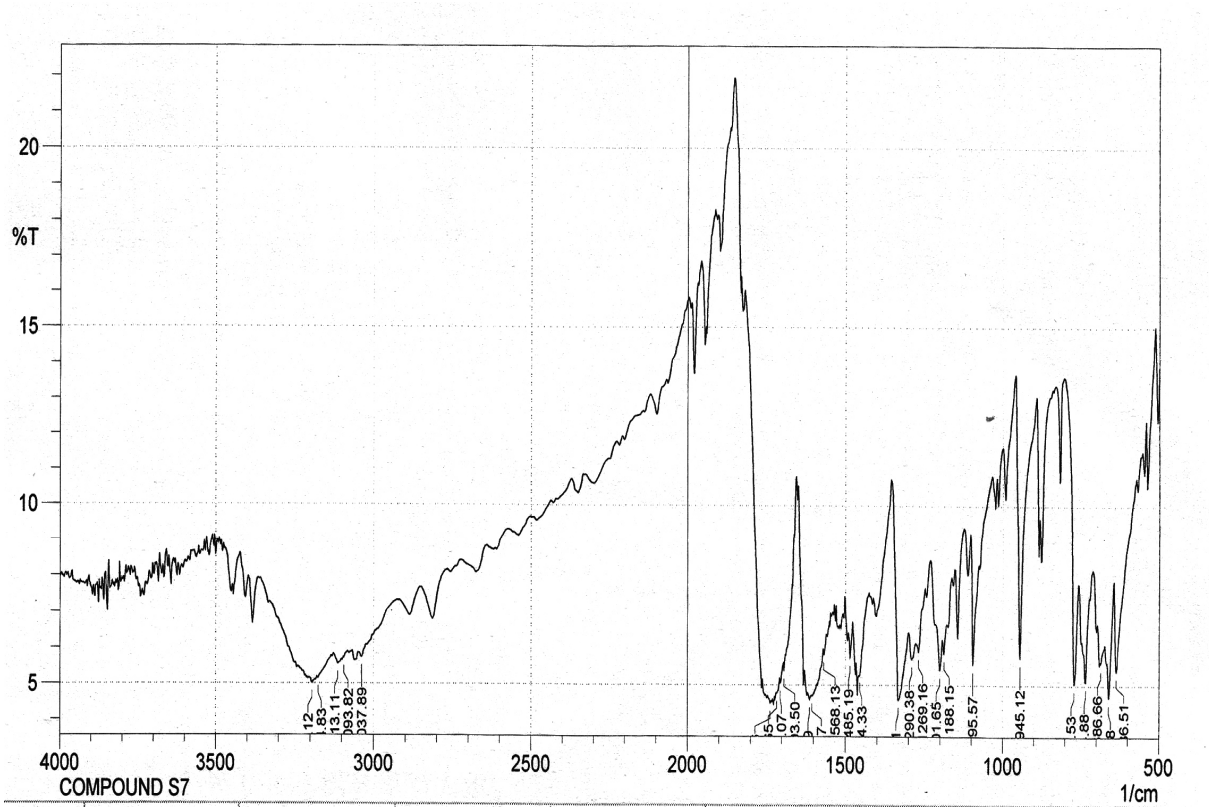

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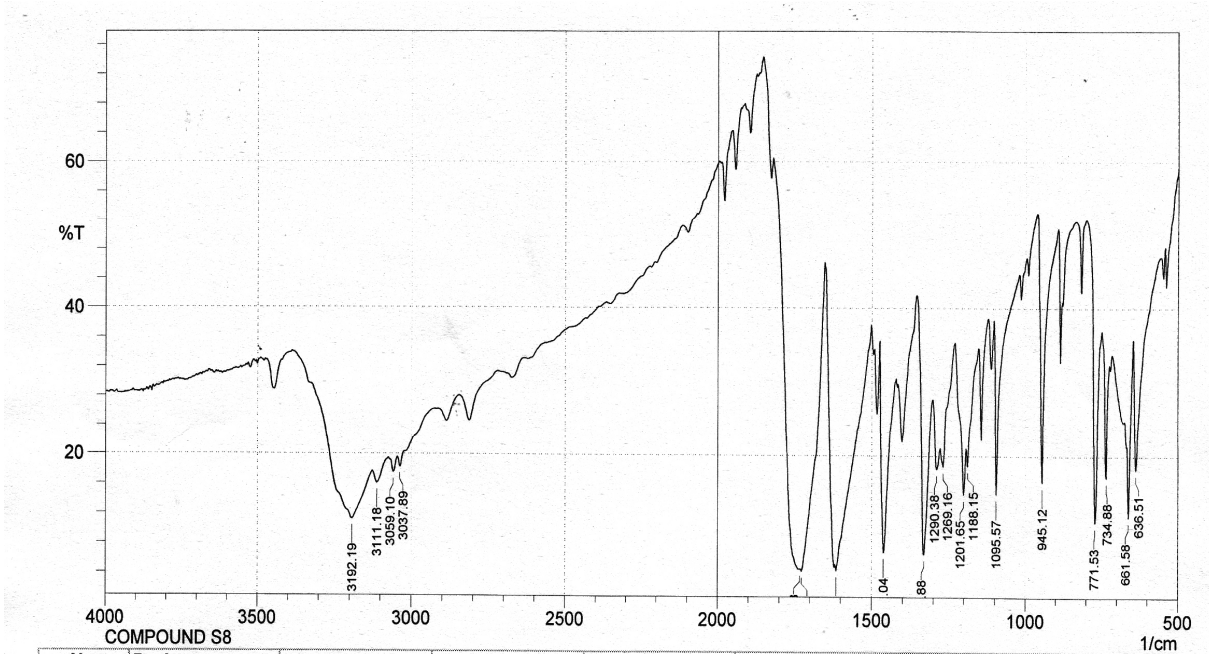

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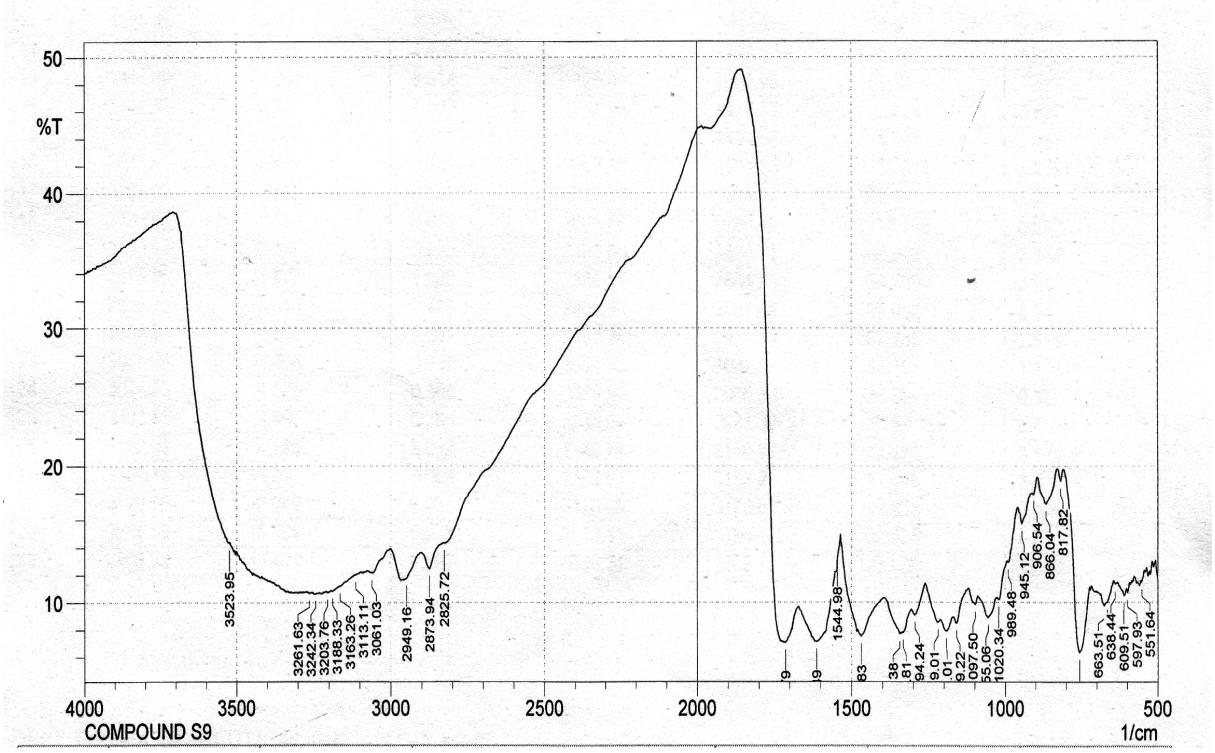

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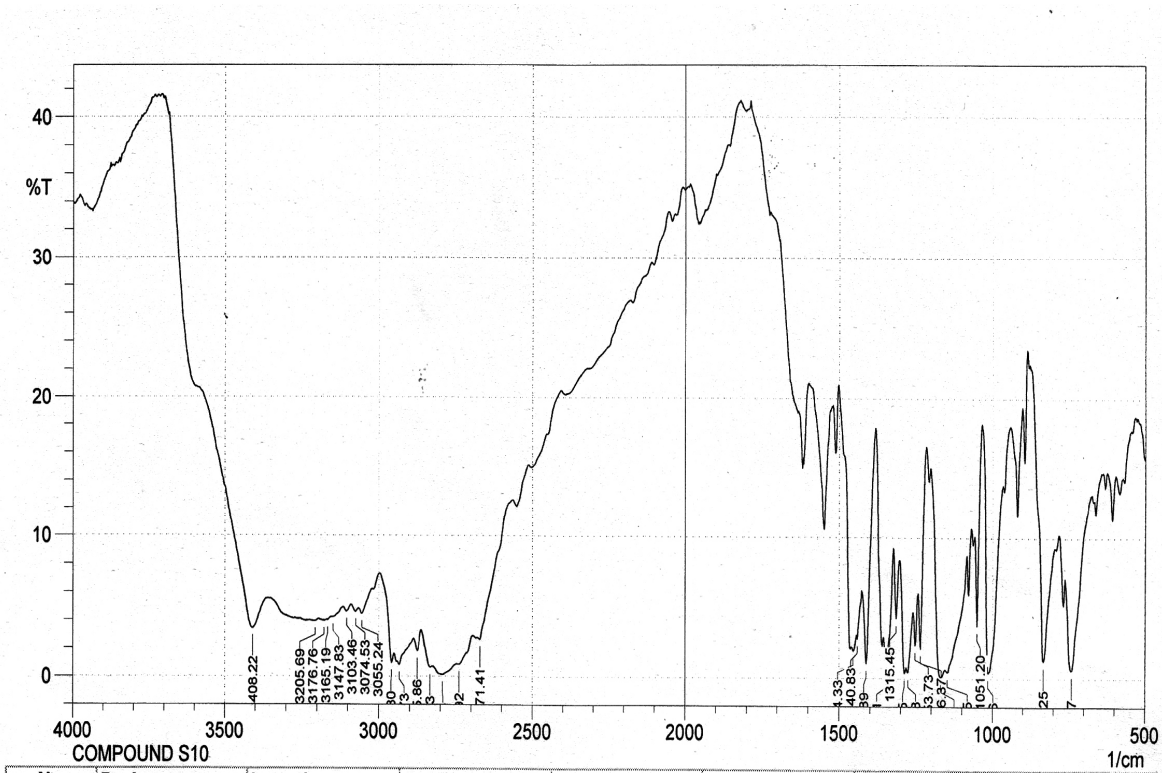
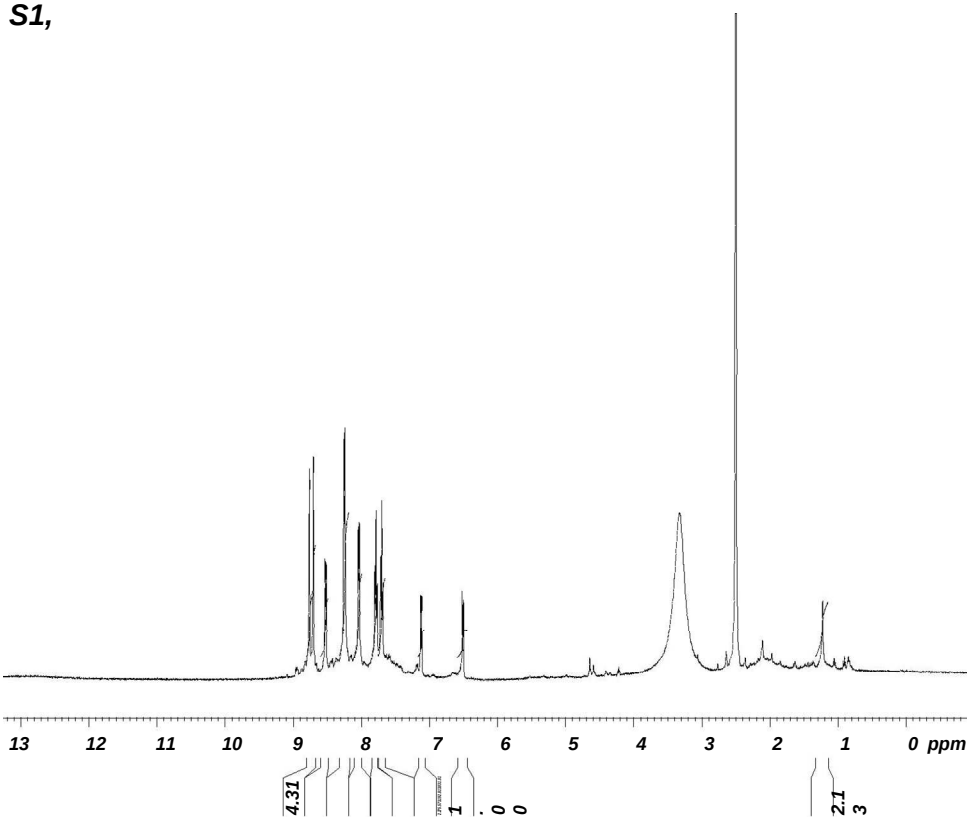


NMR SPECTROSCOPY

S1,



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NAME Feb10−2014
EXPNO 9
PROCNO 1

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PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
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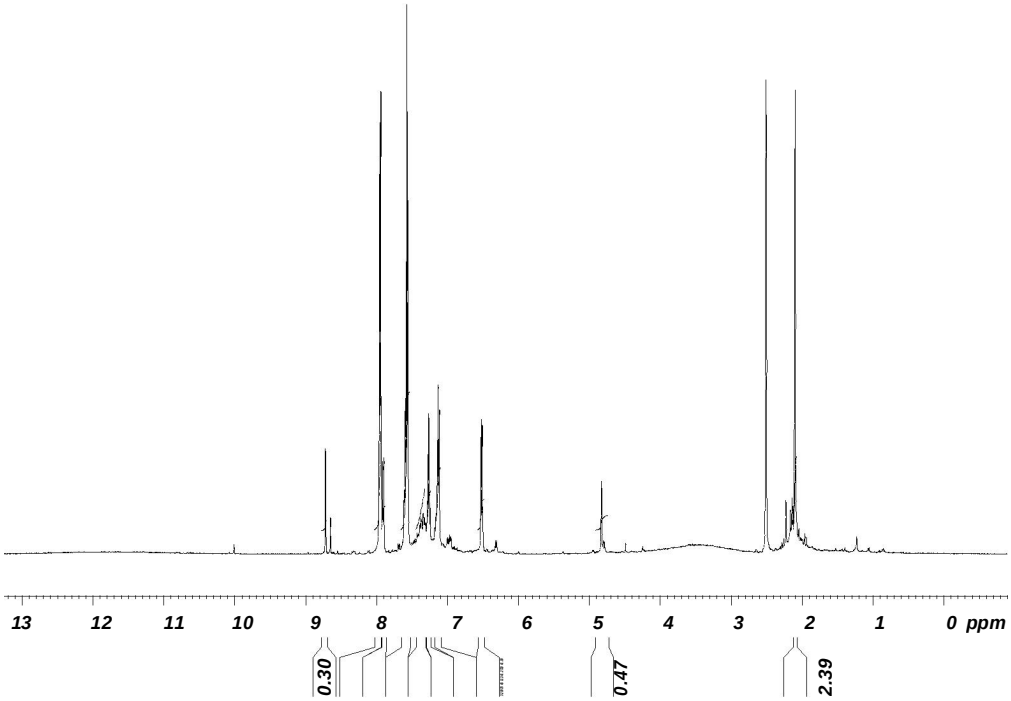
SF 500.1300039 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S2,



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NAME Feb10- 2014
EXPNO 4
PROCNO 1

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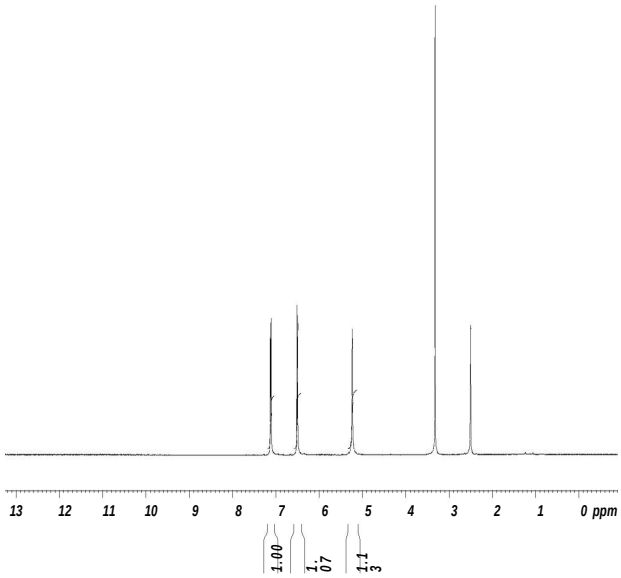
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 - Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S3,



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NAME Feb10−2014
EXPNO 26
PROCNO 1

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Time 4.47
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PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 299.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
SI 32768
SF 500.1300036 MHz
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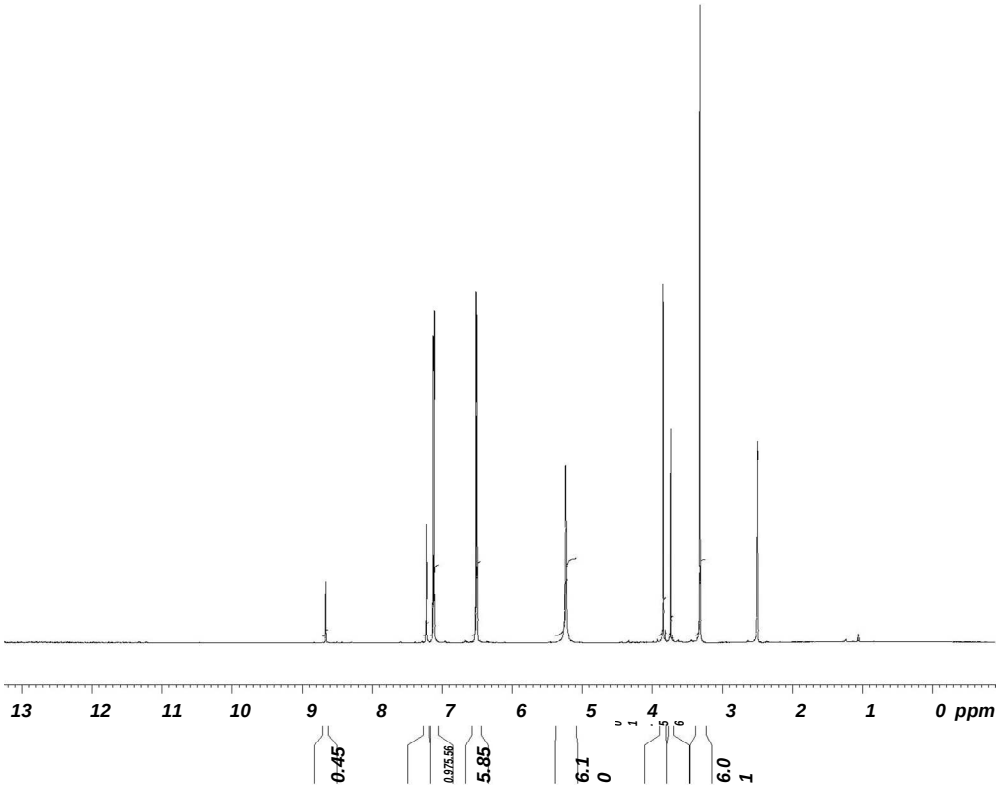
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S4,



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NAME Feb10−2014
EXPNO 13
PROCNO 1

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PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
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SF 500.1300042 MHz
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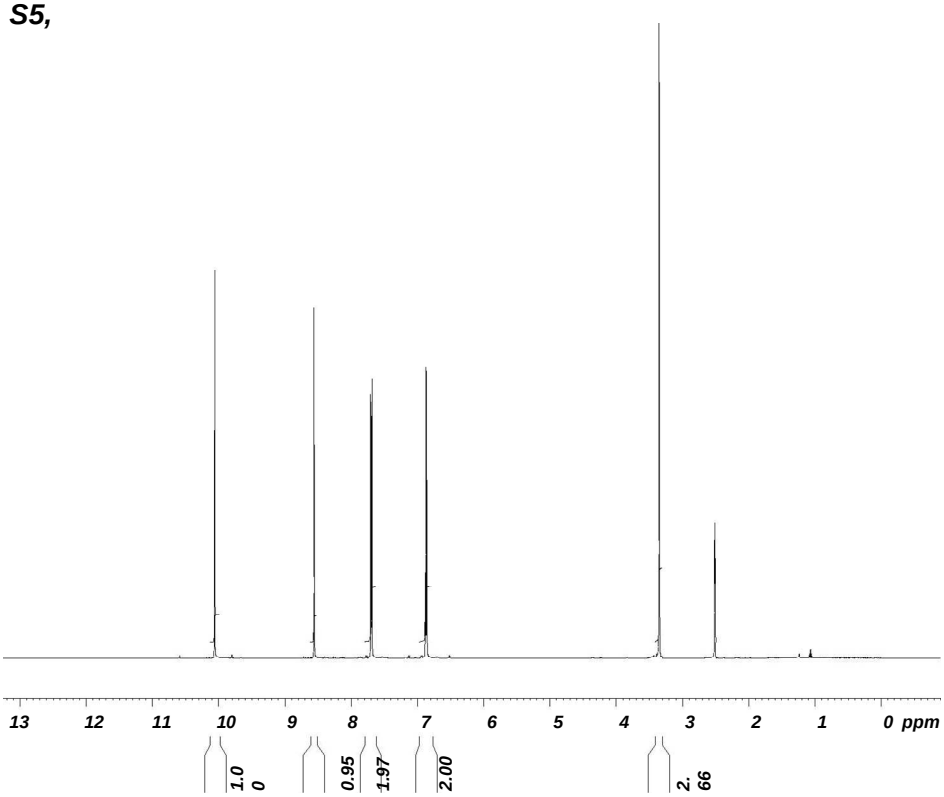
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S5,



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NAME Feb10−2014
EXPNO 12
PROCNO 1

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PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
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FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 203
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
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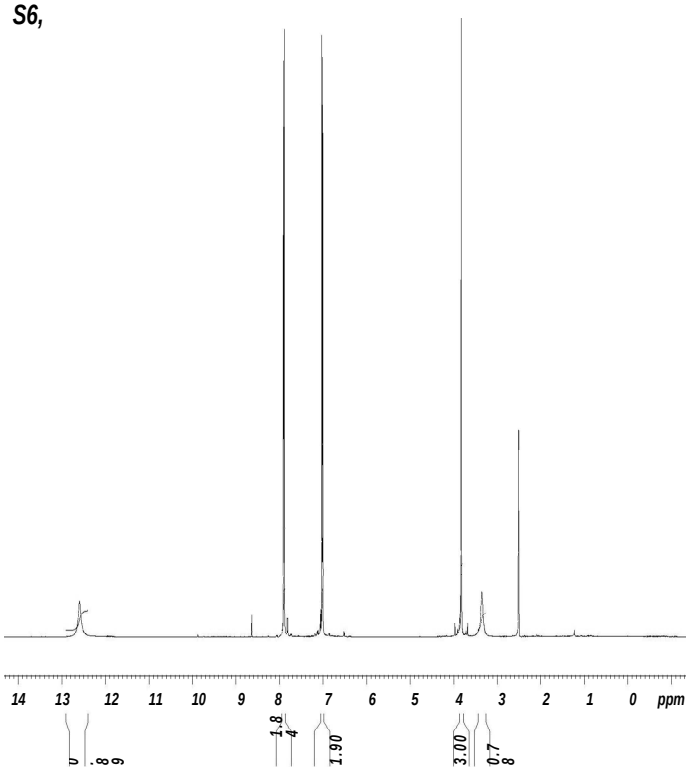
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S6,



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PROCNO 1

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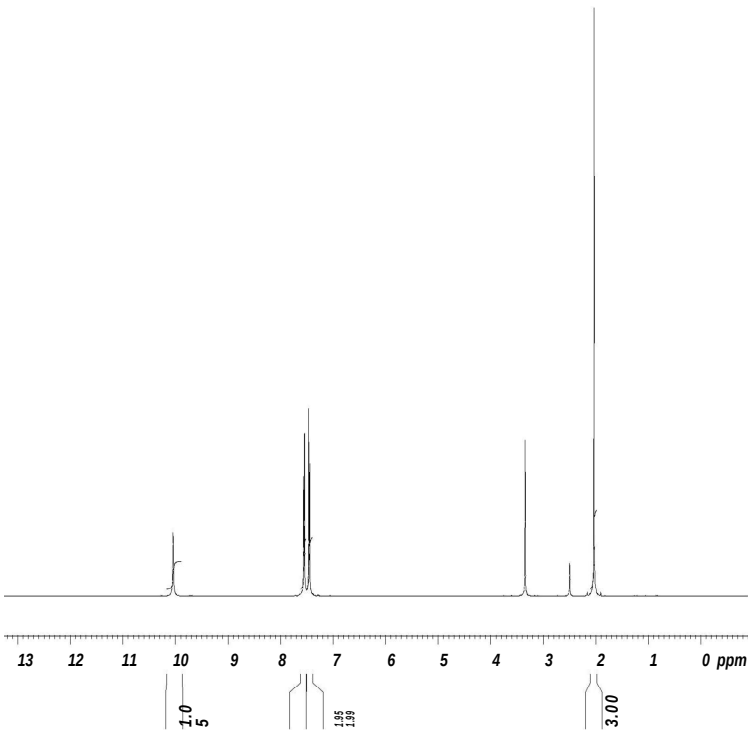
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 181
DW 48.400 usec
DE 6.50 usec
TE 299.3 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
SI 32768
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S7,



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NAME Feb10−2014
EXPNO 7
PROCNO 1

F2 − Acquisition Parameters
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Time 21.56
INSTRUM spect
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PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 161
DW 48.400 usec
DE 6.50 usec
TE 300.1 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
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SF 500.1300039 MHz
WDW EM
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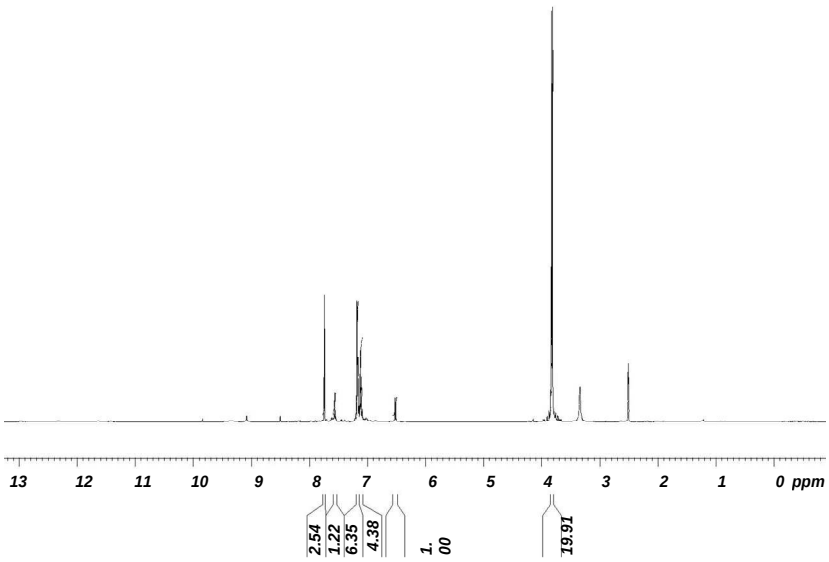
LB 0.30 Hz
GB 0
PC 1.00

S8,



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NAME Feb10−2014
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PROCNO 1

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Time 21.39
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PROBHD 5 mm PABBO BB−
PULPROG zg30
TD 32768
SOLVENT DMSO
NS 32
DS 2
SWH 10330.578 Hz
FIDRES 0.315264 Hz
AQ 1.5860212 sec
RG 181
DW 48.400 usec
DE 6.50 usec
TE 299.9 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 10.65 usec
PL1 0.00 dB
PL1W 23.53637505 W
SFO1 500.1330885 MHz

F2 − Processing parameters
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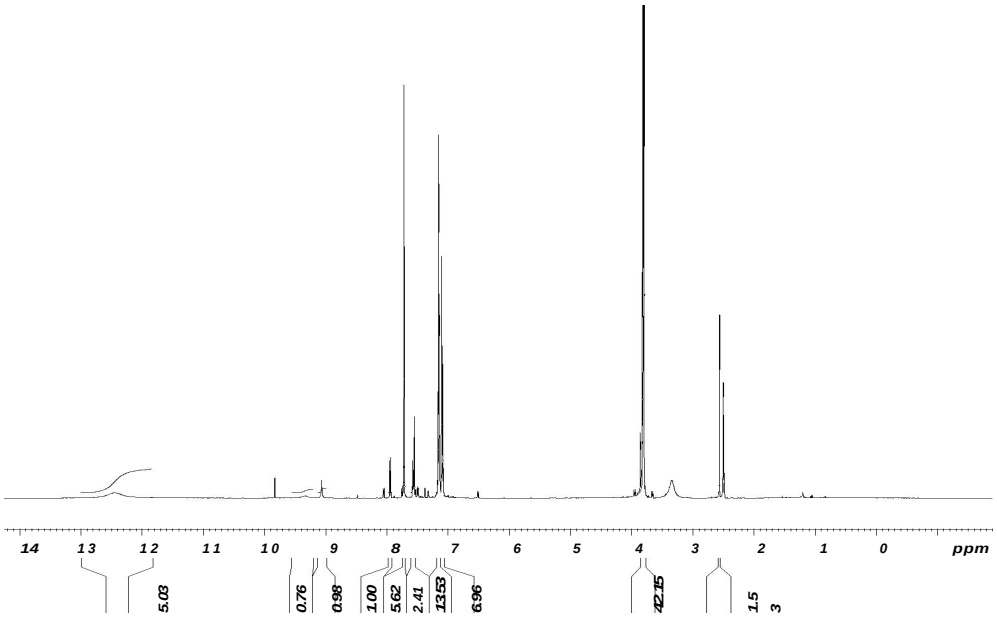
SF 500.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S 9,

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PROCNO                 1

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PROBHD    5 mm PABBO BB-
PULPROG           zg30
TD                32768
SOLVENT            DMSO
NS                    32
DS                     2
SWH           10330.578 Hz
FIDRES         0.315264 Hz
AQ            1.5860212 sec
RG                   144
DW              48.400 usec
DE               6.50 usec
TE               299.7 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1              10.65 usec
PL1               0.00 dB
PL1W         23.53637505 W
SFO1        500.1330885 MHz

F2 - Processing parameters
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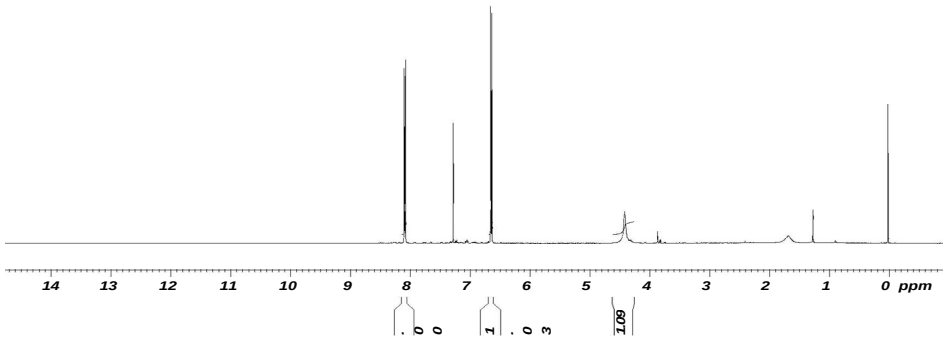
S10.

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PROCNO                 1

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Time               18.17
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    32
DS                     2
SWH            10330.578 Hz
FIDRES         0.315264 Hz
AQ             1.5860212 sec
RG                   203
DW                48.400 usec
DE                 6.50 usec
TE                296.5 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                10.65 usec
PL1                0.00 dB
PL1W         23.53637505 W
SFO1         500.1330885 MHz

F2 - Processing parameters
SI                 32768
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WDW                   EM
SSB                    0
LB                  0.30 Hz
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PC                  1.00
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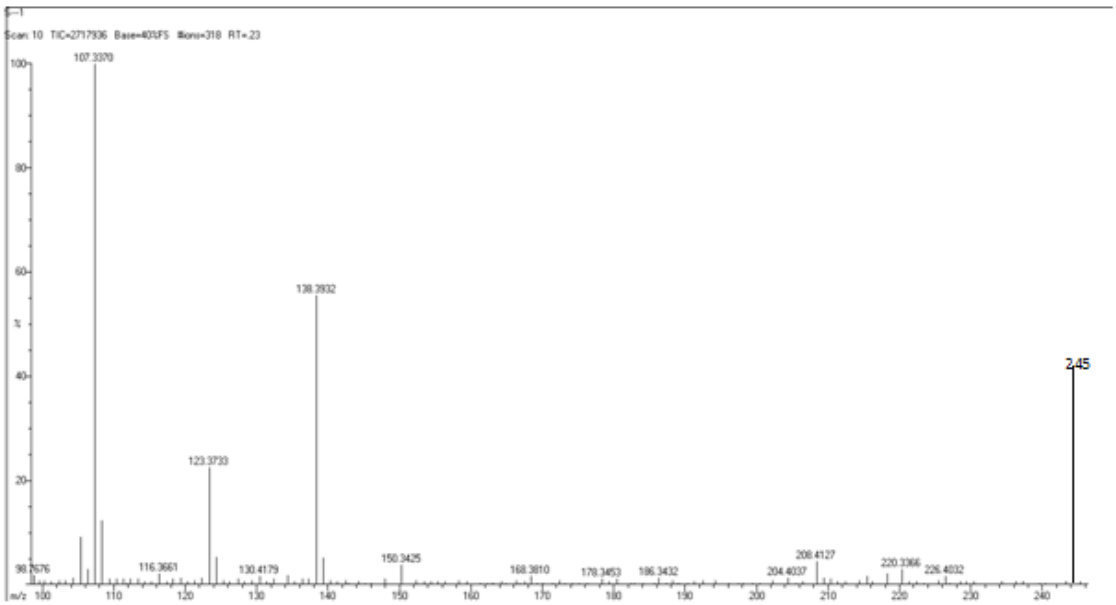
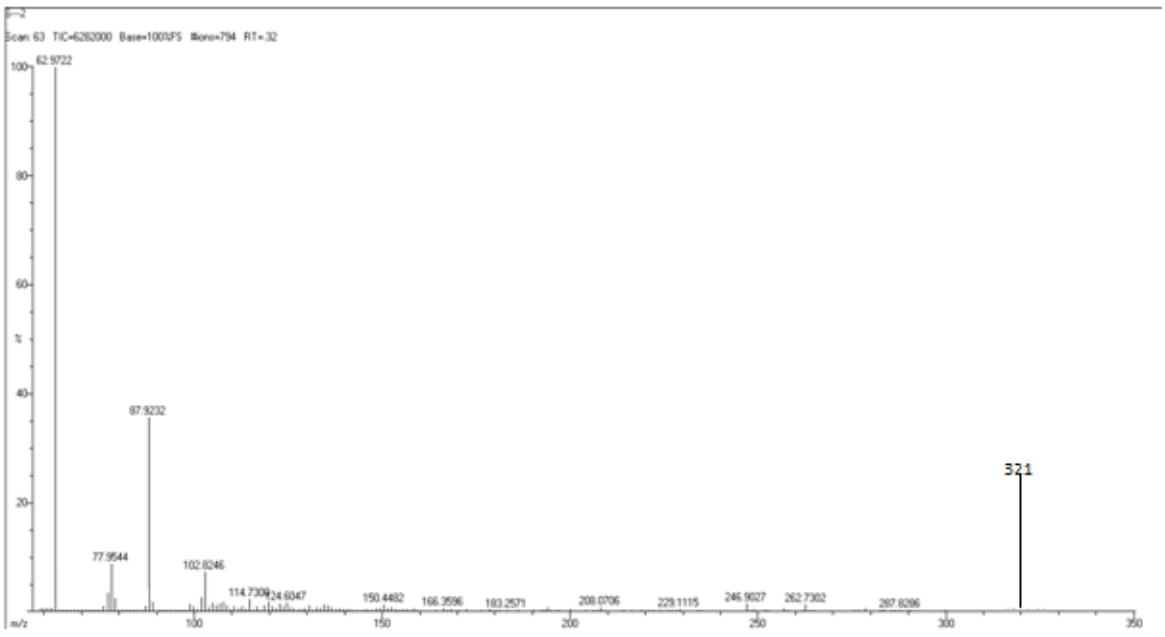
MASS SPECTROSCOPY

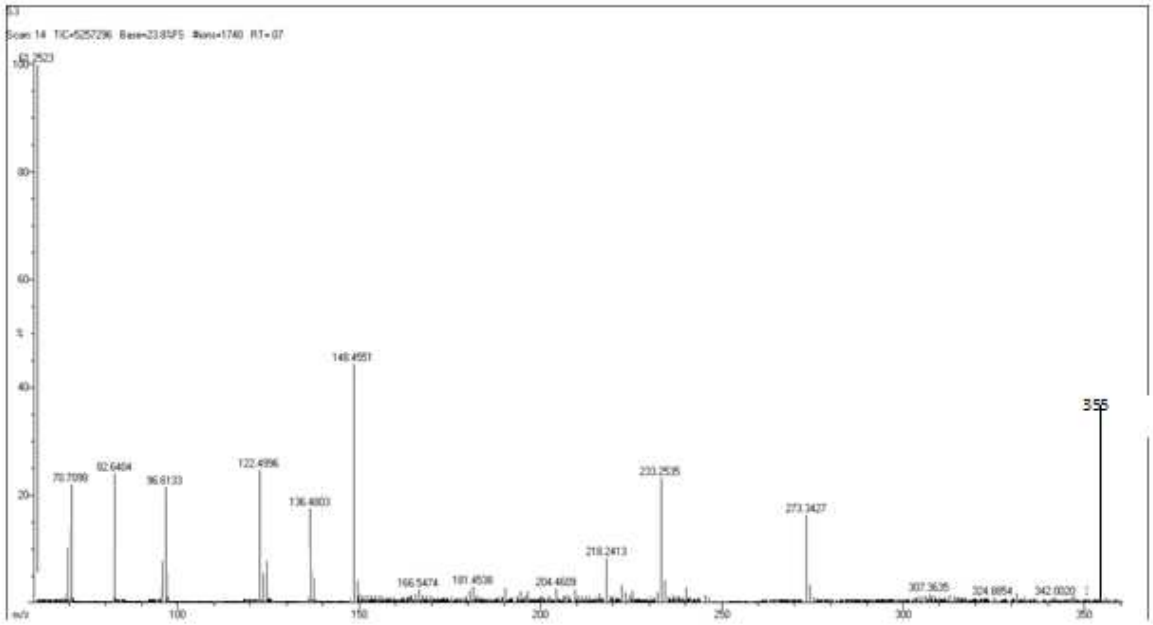
COMPOUND S1



COMPOUND S2



COMPOUND S3



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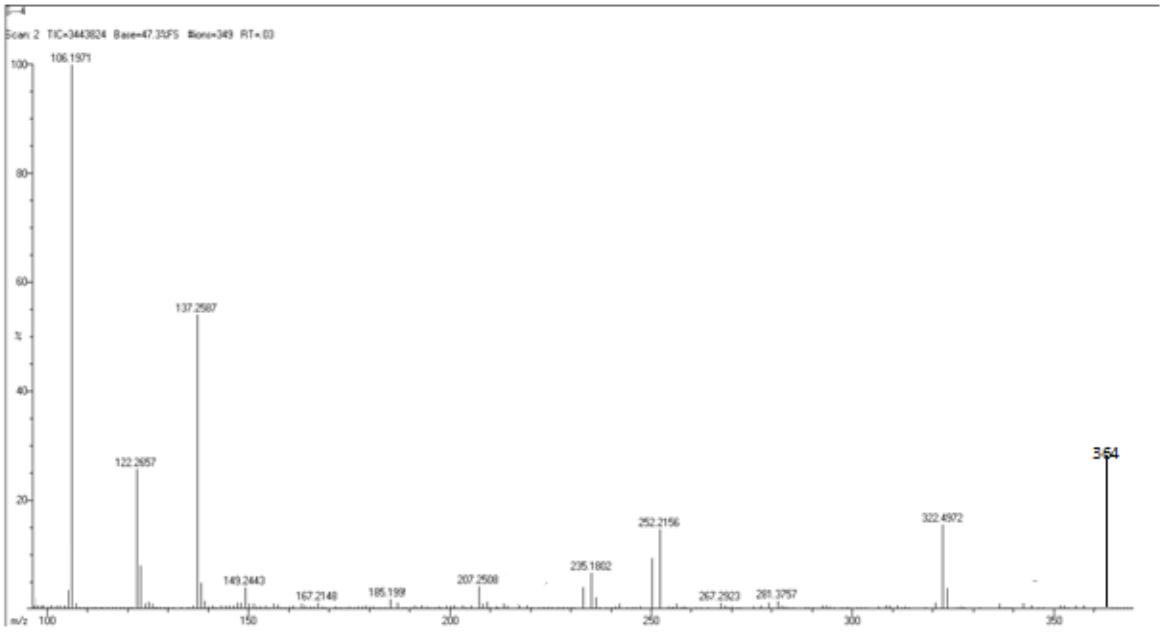
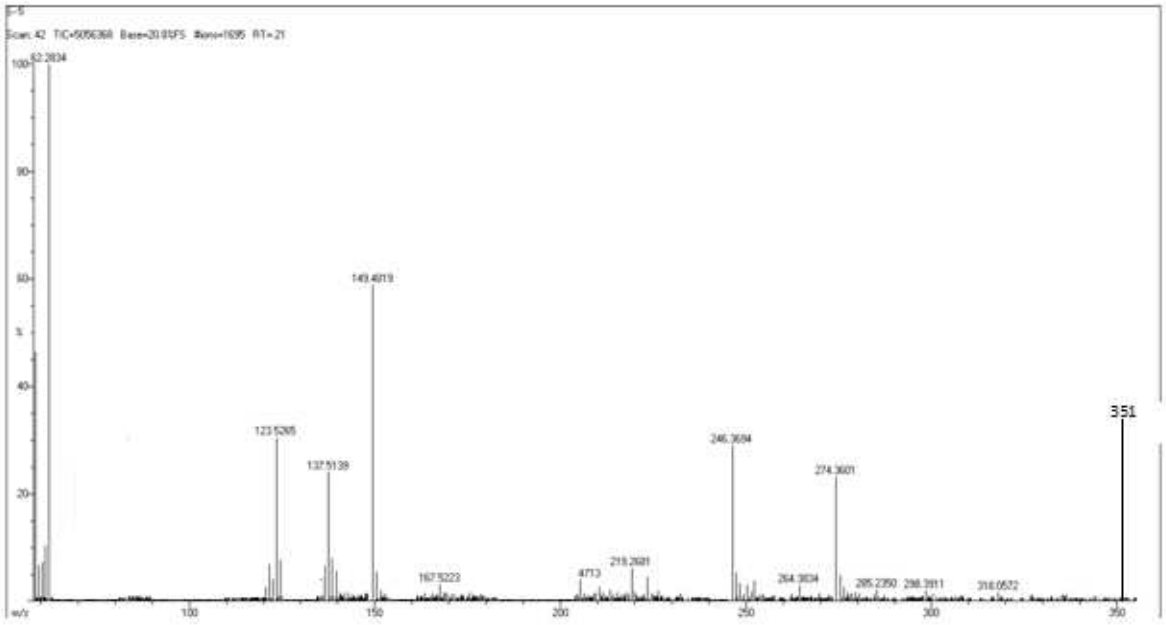


COMPOUND S5



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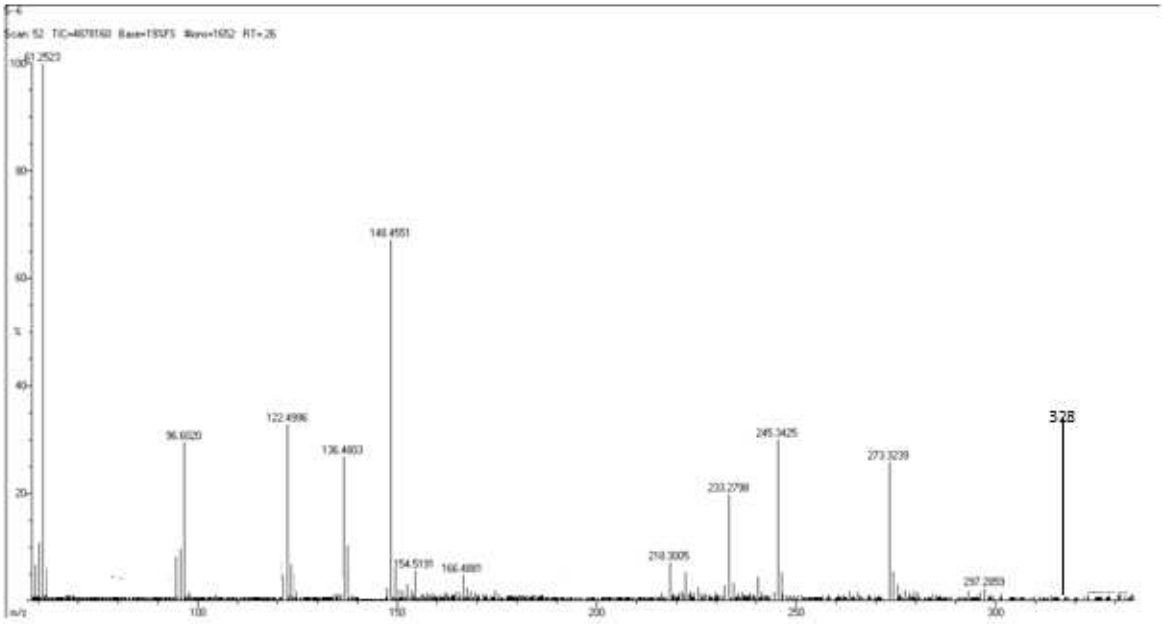

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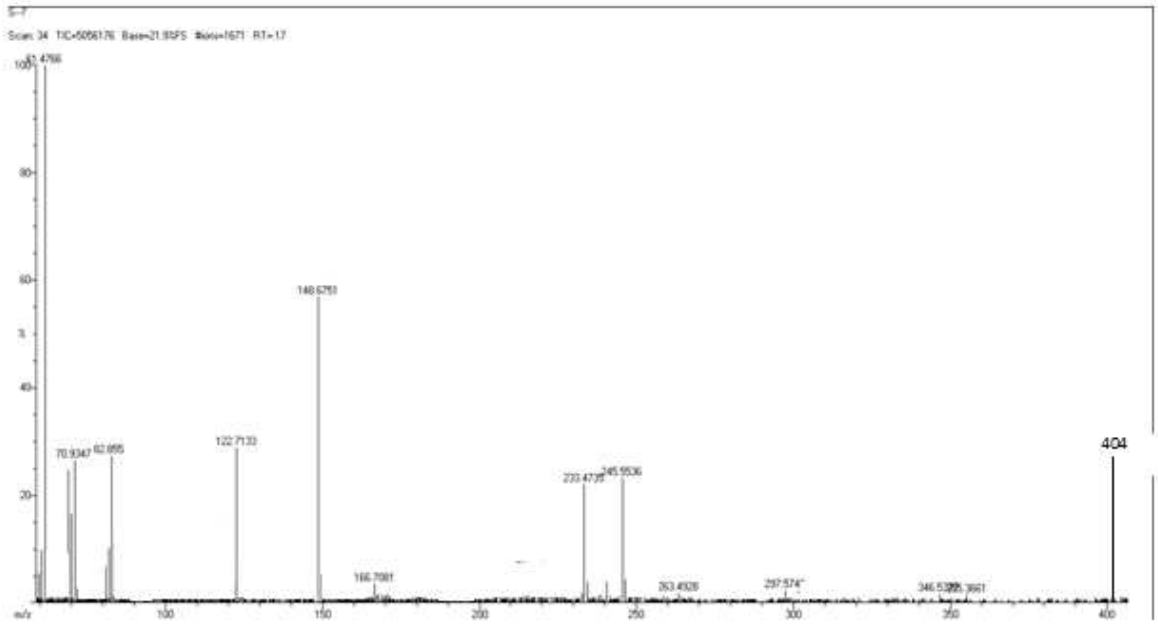
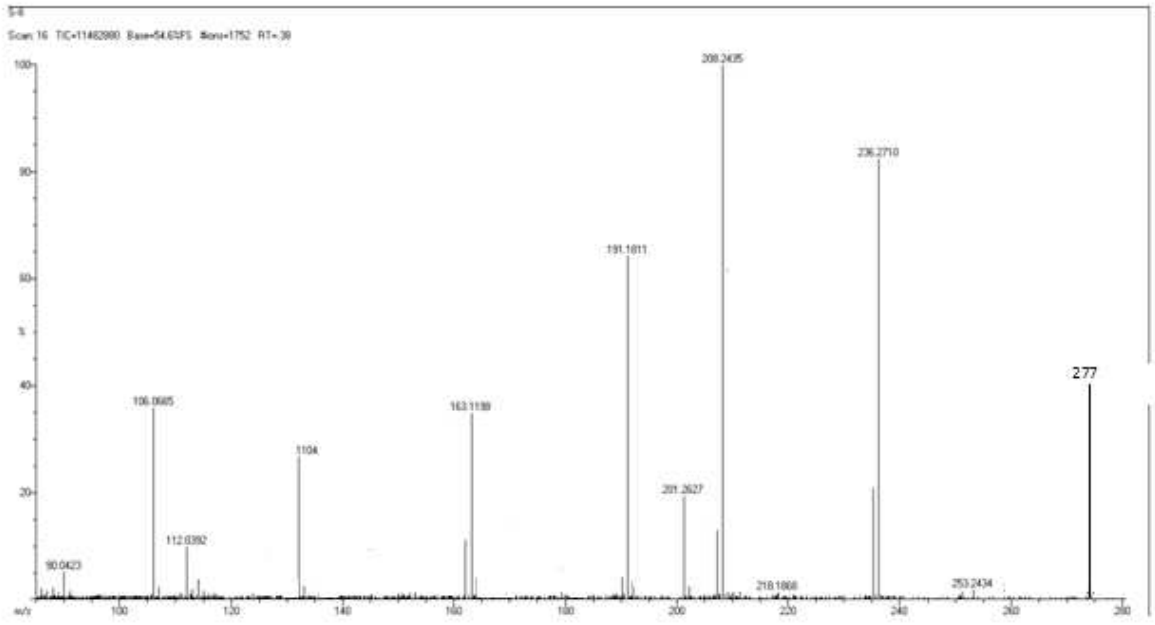


COMPOUND S8



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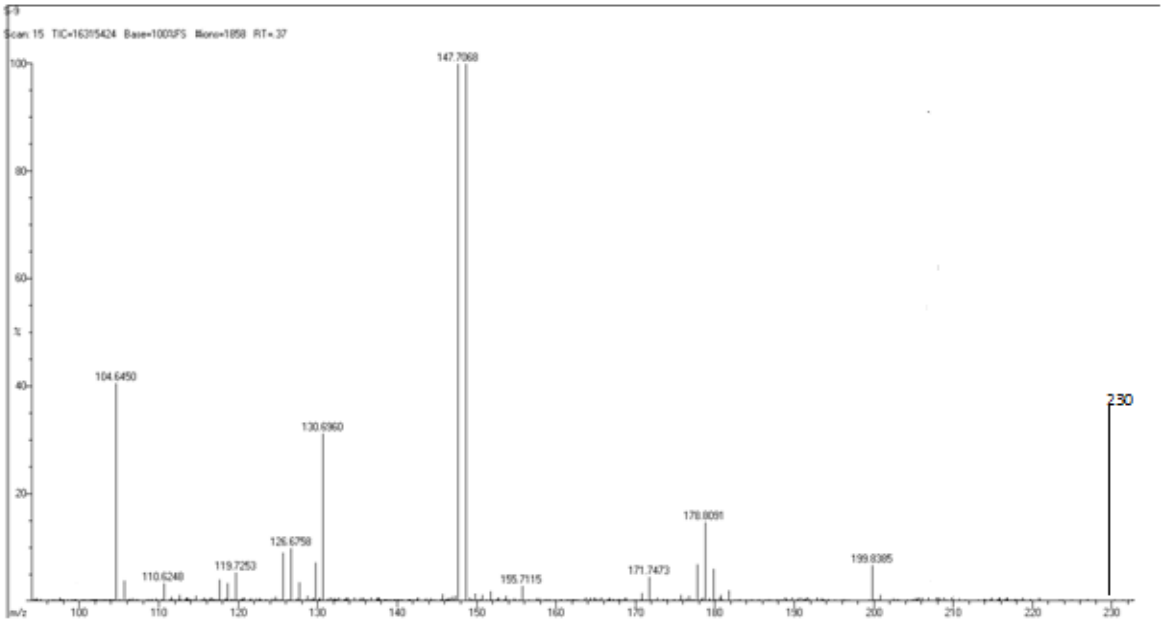
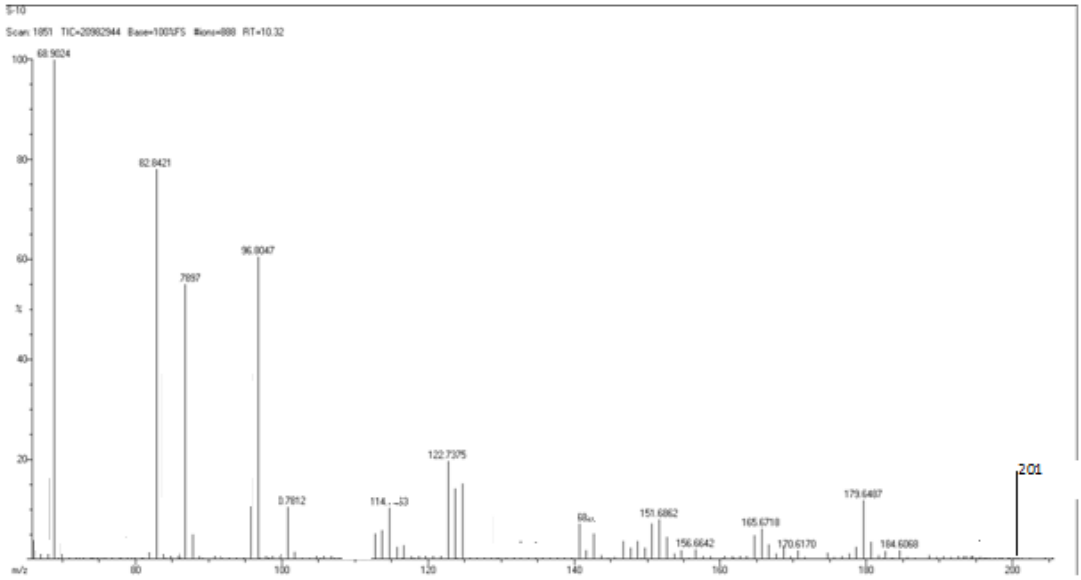


COMPOUND S10



BIOLOGICAL EVALUATION

ANTI OXIDANT ACTIVITY[11,12]

Principle:

The free radical scavenging activity of synthetic drug against hydrogen peroxide was determined by using the method of Ruch et al 1989[70] the principle was based on the capacity drug to decompose the hydrogen peroxide to water.

Reagent:

1] 6% hydrogen peroxide diluted with water in ratio of 1:10

2] 0.1 m phosphate buffer PH[7.4]

Procedure:

Synthetic compound was dissolved in ethanol to get a stock solution containing 1mg/1ml various quantities of the stock solution were added to 3.8 ml of 0.1 m phosphate buffer solution was added and the absorbance was measured at 230 nm after 10 minutes. The reaction without sample was used as blank ascorbic acid was used as standard .the percentage inhibition of hydrogen peroxide was calculated using the formula.

$$= \frac{[\text{A control} - \text{A sample}]}{\text{A control}} \times 100$$

The concentration of compound to produce 50% inhibition was found using linear regression analysis and results obtained are presented.

INVITRO ANTICANCER[48]

METHODOLOGY

Cell line

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of fivesample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

IN VITRO- ANTIBACTERIAL ACTIVITY[22]

TEST CONCENTRATION:

- 100µg/ml
- 200µg/ml

ORGANISM USED:

- Staphylococcus aureas
- Salmonella typhi

SOLVENT USED:

- DMSO

STANDARD DRUG:

- Amikacin

MEDIA PREPARATION[21]

MULLER- HINTON AGAR MEDIUM:

INGREDIENTS:

Beef infusion	-	300ml
Casein Hydrolysate	-	17.5g
Starch	-	1.5g
Agar	-	10g
Distilled water	-	1litre

PROCEDURE:

Emulsify the starch in a small amount of cold water, pour into the beef infusion and add the casein hydrolysate and the agar. Make up the volume to 1litre with distilled water. Dissolve the constituents by heating gently at 100°C with agitation. Filter if necessary. Adjust the pH to 7.4.Dispense in screw-capped bottles and sterilized by autoclaving at 121°C for 20minutes and pour plates.

PREPARATION OF ANTIBACTERIAL SOLUTION:

All the test compound were dissolved in dimethyl sulfoxide and taken at two concentration for testing antibacterial activity. The compounds were diffuse into the medium produced a concentration gradient. After the incubation period, the zone of inhibition were measured in mm.

EXPERIMENTAL PROCEDURE:

The plates were inoculated by dipping a sterile swab into inoculums. The inoculation was dried at room temperature in aseptic condition. Ditch the bore in plate, to this bore add prepared antibacterial solution. These plates were placed in an incubator at 37°C within a few minutes of preparation. After 48 hours of incubation the diameter of zone of inhibition was measured and reading observed in millimeter.

RESULTS AND DISCUSSION

LIPINSKIS RULE BY CHEMDOODLE

Table.No:1

Code	M.W	M.R cm^3/mol	H bond acceptor	H bond donor	Logp	No.of.criteria
Rule	**<500**	**<150**	**<10**	**<5**	**<5**	**Atleast 3**
S1	245.2	70.3	4	1	1.9	ALL
S2	321.3	94.8	4	1	3.5	ALL
S3	355.8	99.8	4	1	4.1	ALL
S4	364.4	109.1	5	1	4.1	ALL
S5	351.3	101.3	5	1	3.8	ALL
S6	328.3	100.9	3	0	4.3	ALL
S7	404.4	123.4	2	1	6.4	ALL
S8	277.2	80.7	2	0	1.2	ALL
S9	230.2	66.7	3	0	2.4	ALL
S1O	215.2	66.7	2	2	3.5	ALL

PHYSICAL DATA

TABLE-2

CODE	MOLECULAR FORMULA	MOLECULAR WEIGHT	I.U.P.A.C NAME
S1	C13H15N3O2	245.277	1-(Piperazine-1-ylmethyl)-1H-indole 2,3 dione
S2	C19H19N3O2	321.373	1-(phenyl (piperazine-1-yl methyl)-1H –indole 2,3 dione
S3	C19H18N3O2CL	355.818	1-(4-chlorophenyl)(piperazine-1-yl methyl)-1H- indole2,3 dione
S4	C21H24N4O2	364.440	1-[(4-dimethylaminopiperazine-yl-methyl)-1H-indole 2,3 dione
S5	C20H21N303	351.399	1-[(4methoxyohenyl)(piperazine-yl methyl)-1H- indole2,3 dione
S6	C21H16N2O2	328.3639	1-[(diphenylamino) methyl]-1H indole 2,3 dione
S7	C27H20N2O2	404.459	1-[diphenylamino,phenyl]-yl methyl)-1H-indole 2,3 dione
S8	C16H11N3O2	277.277	1-(1H-benzimidazole-yl methyl)-1H-indole 2,3 dione
S9	C13H14N2O2	230.262	1-(pyrrolidine-1-yl methyl)-1H-indole-2,3 dione
S10	C12H15N3	201.267	3-(piperazine-1-yl methyl)-1H-indole

PHYSICAL DATAS

TABLE-3

Compounds	Nature of Crystals	Melting Point	% Yield	Soluble in
S1	Pale orange solid	197	80	DMSO
S2	Orange solid	212	72	DMSO
S3	Pale yellow solid	204	75	DMSO
S4	Brick red solid	220	82	DMSO
S5	Red solid	206	76	DMSO
S6	Dark red soid	216	82	DMSO
S7	Red solid	230	74	DMSO
S8	Brick red solid	202	80	DMSO
S9	Black colour solid	210	75	DMSO
S10	White solid	180	70	DMSO

THIN LAYER CHROMOTOGRAPHY

TABLE-4

S.NO	COMPOUND	RF-VALUE
1	S1	0.37
2	S2	0.34
3	S3	0.41
4	S4	0.42
5	S5	0.39
6	S6	0.44
7	S7	0.46
8	S8	0.43
9	S9	0.38
10	S10	0.47

SOLVENT SYSTEM: **BENZENE: METHANOL**

RATIO: **9:1**

ELEMENTAL COMPOSITION

TABLE-5

Compounds	Elemental Composition in Percentage				
	C	H	N	O	Cl
S1	63.66	6.16	17.13	13.05	
S2	71	5.96	13	9	
S3	64.13	5.10	11.81	8.99	9.96
S4	69.21	6.66	15.37	8.78	
S5	68.36	6.02	11.96	13.66	
S6	76.81	4.91	8.53	9.74	
S7	80.18	4.98	6.93	7.91	
S8	69.31	4.00	15.15	11.54	
S9	67.81	6.13	12.17	13.90	
S10	71.61	7.51	20.88		

IR SPECTROSCOPY DATAS

TABLE-6

COMPOUND	FUNCTIONAL GROUP	FREQUENCIES CM^{-1}
S1	C=O [str] C-N [str] C=C [str] N-H [str] CH Stretching in benzene	1739 1350 1612 3192 2956
S2	C=O[str] C-N [str] C=C [str] N-H [str] CH stretching in benzene	1726 1330 1620 3192 3037
. S3	C=O [str] C-N [str] N-H [str] C=C [str] C-CL [str] CH stretching in benzene	1705 1332 3346 1612 802 2960
S4	C=O [str] C-N [str] N-H [str] C=C [str] C-C [str]	1728 1330 3192 1616 1095
S5	C=O [str] C-N [str] C=C [str] N-H [str] C-OCH3 [str]	1726 1330 1616 1201 1095

IR SPECTROSCOPY DATAS

TABLE-7

COMPOUND	FUNCTIONAL GROUP	FREQUENCIES CM^{-1}
S6	C=O [str] C-N [str] C=C [str] N-H [str] CH Stretching in benzene	1728 1188 1614 1330 2960
S7	C=O[str] C-N [str] C=C [str] C - C[str] CH stretching in benzene	1705 1201 1693 771 2930
S8	C=O [str] C-N [str] C=N [str] C-C [str] CH stretching in benzene	1726 1330 1614 1201 2920
S9	C=O [str] C-N [str] C=C [str] C-C [str] CH stretching in benzene	1712 1332 1612 1192 2960
S10	C-N [str] C-C [str] N-H [str] CH stretching in benzene	1346 1168 3408 2958

NMR SPECTROSCOPY DATAS

TABLE-8

CODE	TYPES OF PROTON	OBSERVED VALUE In PPM
S1	m 4H in Ar H s 2H in CH2 s 1H in NH	8.1 3.3 2.7
S2	m 4H in Ar H s 1H in NH	7.5 2.3
S3	m 4H in Ar H s 1H in NH s 1N in CH	7.3 2.6 3.3
S4	m 8H in Ar H d 6H in CH3 s 1H in NH	7.3 6.5 3.3
S5	m 8H in Ar H s 3H in OCH3 s 1H in NH	7.8 3.4 2.6
S6	m 14H in Ar H s 2H in CH2	7.2 3.8
S7	m 19H in Ar H s 1H in CH	7.5 3.3
S8	m 9H in Ar H d 2H in CH2	7.2 3.8
S9	m 10H in CH2 m 4H in Ar H	3.9 7.7
S10	m 4H in Ar H s 2H in NH s 2H in CH2	8.1 4.5 3.8

MASS SPECTROSCOPY DATAS

TABLE-9

SL. NO.	COMPOUND	OBSERVED PEAK
1	S1	245
2	S2	321
3	S3	355
4	S4	364
5	S5	351
6	S6	328
7	S7	404
8	S8	277
9	S9	230
10	S10	201

ANTI BACTERIAL ACTIVITY

[ZONE OF INHIBITION AGAINST MICRO ORGANISM]

TABLE-10

COMPOUND	STAPHYLOCOCCUS AUREAS		SALMONELLA TYPHI	
	100µm	200µm	100µm	200µm
S1	19mm	19mm	11mm	13mm
S2	12mm	18mm	17mm	22mm
S3	10mm	15mm	13mm	17mm
S4	14mm	21mm	15mm	21mm
S5	14mm	19mm	17mm	23mm
S6	13mm	15mm	12mm	14mm
S7	16mm	22mm	18mm	25mm
S8	14mm	19mm	17mm	24mm
S9	18mm	19mm	17mm	19mm
S10	16mm	23mm	14mm	21mm
STANDARD	19mm	20mm	17mm	18mm
CONTROL	R	R	R	R

Standard – Amikacin

Control - DMSO

ANTI OXIDANT PROPERTIES

TABLE-11

COMPOUNDS/ CONCENTRATION	% INHIBITION*		
	100µg/ml	200µg/ml	300µg/ml
S1	72.6±0.53	76.4±0.2	69±0.0.28
S2	70.8±0.46	74.3±0.42	76.7±0.31
S3	56.1±0.14	59.8±0.26	64.3±0.29
S4	76.0±0.07	78.7±0.16	79.8±0.24
S5	75.1±0.13	76.8±0.22	78.7±0.21
S6	64.2±0.22	71.2±0.24	73.9±0.13
S7	72.9±0.34	76.8±0.25	79.7±0.22
S8	75.5±0.39	78.0±0.11	83.71±0.3
S9	57.6±0.31	61.7±0.35	64.8±0.16
S10	55.6±0.20	57.9±0.23	59.8±0.27
STD	83.1±0.20	85.2±0.17	87.5±0.23

STD: ASCORBIC ACID

***Mean 3value ±SEM**

CYTO TOXICITY STUDIES (MTT ASSAY)

TABLE-12

COMPOUND	CONCENTRATION (µg/ml)	ABSORBANCE	%CELL VIABILITY
S1	0.1	0.47	101.1
	1	0.45	97.3
	10	0.43	93.2
	50	0.42	83.4
	100	0.40	85.4
	control	0.47	101.1
S3	0.1	0.46	99.7
	1	0.45	97.0
	10	0.44	95.2
	50	0.42	90.9
	100	0.40	85.4
	Control	0.47	101.1
S6	0.1	0.47	100.8
	1	0.46	97.9
	10	0.46	98.5
	50	0.44	94.5
	100	0.38	81.3
	Control	0.47	101.1
S8	0.1	0.47	100.0
	1	0.46	99.0
	10	0.45	95.8
	50	0.41	86.6
	100	0.31	67.7
	Control	0.47	101.1
S9	0.1	0.46	99.6
	1	0.46	99.3
	10	0.45	97.2
	50	0.45	96.4
	100	0.44	93.9
	control	0.47	101.1

Results and Discussion

- The molecular design of synthesized compound were done by using different software.
- The lipinskin rule was predicted for all synthesized compound using CHEMDOODLE. It shows no vialation in basic properties .The results were shown in **Table -1**
- The molecular formula,molecular weight and I.U.P.A.C name were predicted and shown in **Table-2**
- The pecentage yield, melting point, solubility and appearance of the compound are determined and shown in **Table-3**
- The purity of the compounds were checked by TLC and Rf value were calculated. The results were shown in **Table-4**
- Elemental compostion were found and calculated in percentage and results obtained were shown in **Table-5**
- The structure of the synthesized compounds were confirmed by IR spectra NMR spectra and Mass spectra.
- IRspectra interpert value shown in **Table -6,7**
- NMR specctra interpert value shown in **Table -8**
- Mass spectra results were shown in **Table-9**
- All synthesized compounds were screened for their invitro antimicrobial, antioxidant and anti cancer activity
- The antibacterial activity was performed against staphylococcus aureas,salmonella typhi. The zone of inhibition was performed by cup-plate method and results were measured in milimeter shown in **Table-10**

- The graphical representation compound were shown and compared with the standard amikacin
- All synthesized compound were tested for invitro anti oxidant activity by hydrogen peroxide method in different concentration and compared with the standard ascorbic acid. The result are shown in **Table-11**
- All of the newly obtained compound were tested for invitro anti cancer activity by MTT Assay method in different concentration and compared with the standard diclofenac. The results are shown in **Table-12**

SUMMARY AND CONCLUSION

The present study describes the synthesis of novel series of 1-substituted isatin derivatives by mannich reaction this methodology offers the wide advantages of the existing procedure available for the synthesis of isatin derivatives.

The purity of the synthesised compounds were analysed by thin layer chromatography. The structure of the synthesised compound has been elucidated by infra red ,nuclear magnetic resonance,mass spectroscopy.

The compound S5,S7,S8 shown potent antibacterial activity against salmonella typh.i the compound S7,S10 shown best anti bacterial activity against staphylococcus aureas both are compared to standard amikacin.

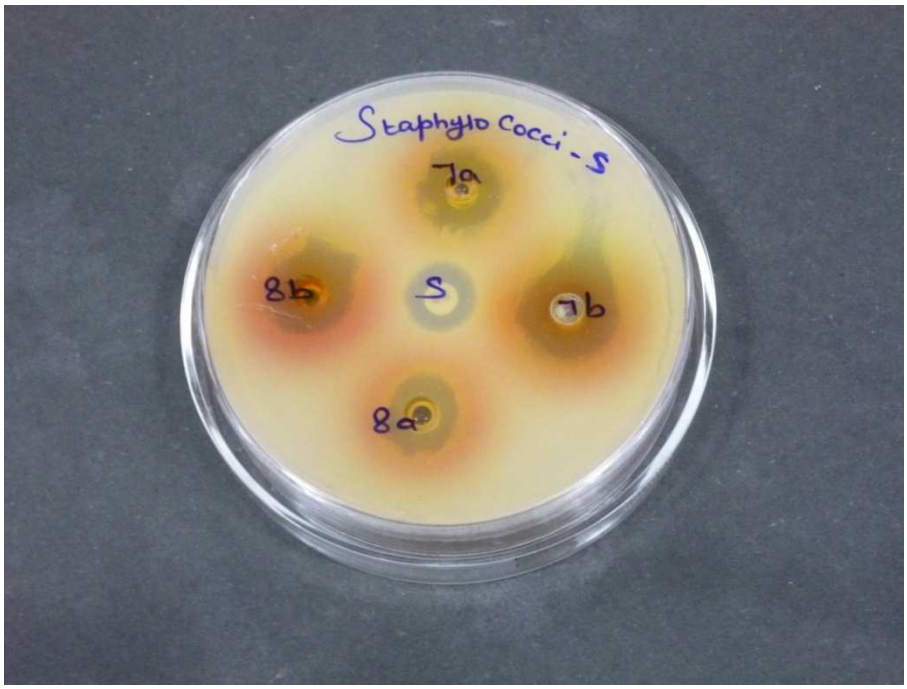
The new data might be helpful in the future development of isatin analogue as novel broad spectrum antibacterial agent.

The compound S4,S7,S8 Shown good anti oxidant properties it has identified by the assay of hydrogen peroxide method.

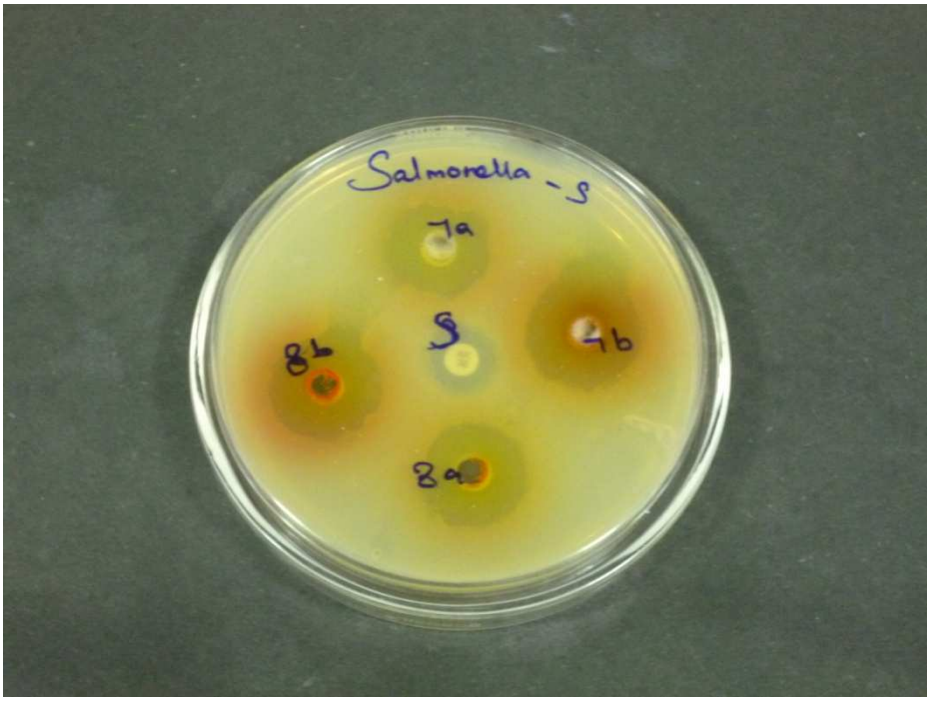
The anti cancer activity of the synthesised compounds were screened by using MTT assay in cervical cancer cell line, from this result compound S8 having 67%cell viability. so the compound S8 shown significantly less cytotoxic activity.

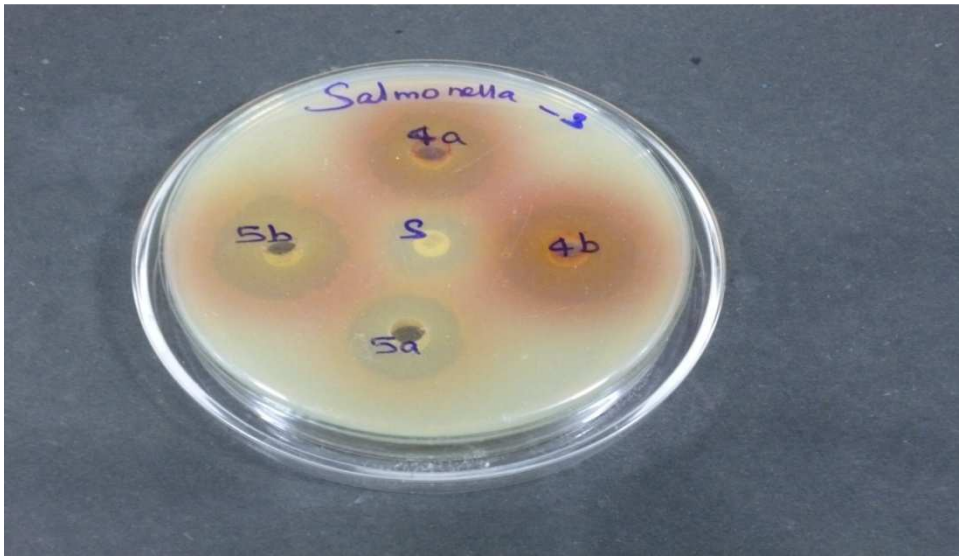
Ultimately i would like to deliver the compound S8 (**1-[1H-benzimidazole-1-yl methyl]-1H-indole-2, 3 –dione)**having three activities like ant oxidant activity, anti bacterial activity and cytotoxic activicty, in future compound S8 should be modified by chemical reaction when it gives potent and effective molecule.

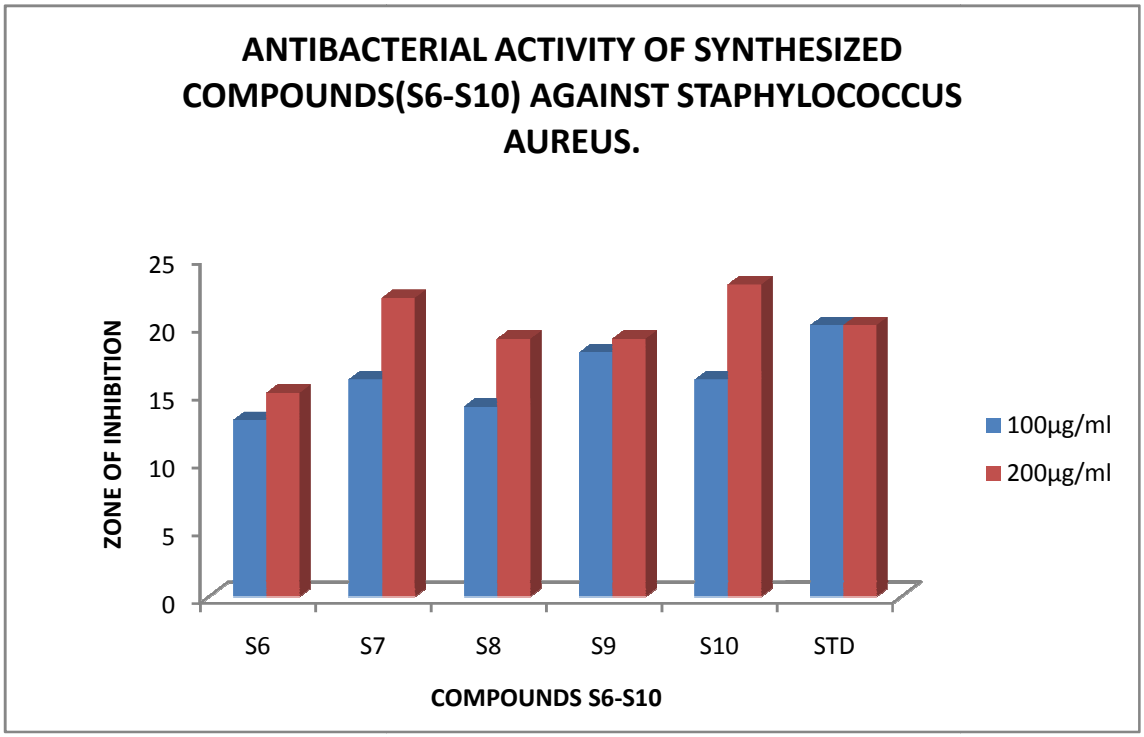
ANTIBACTERIAL ACTIVITY AGAINST STAPHYLOCOCCUS AURES

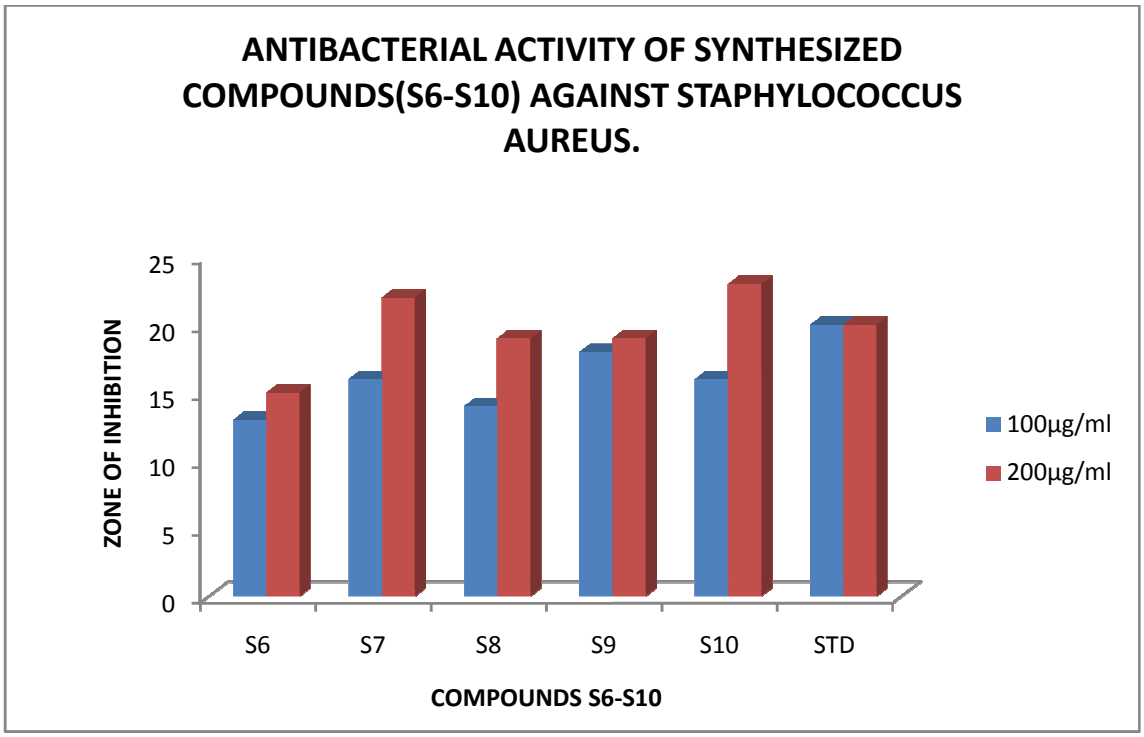


ANTIBACTERIAL ACTIVITY AGAINST SALMONELLA TYPHI

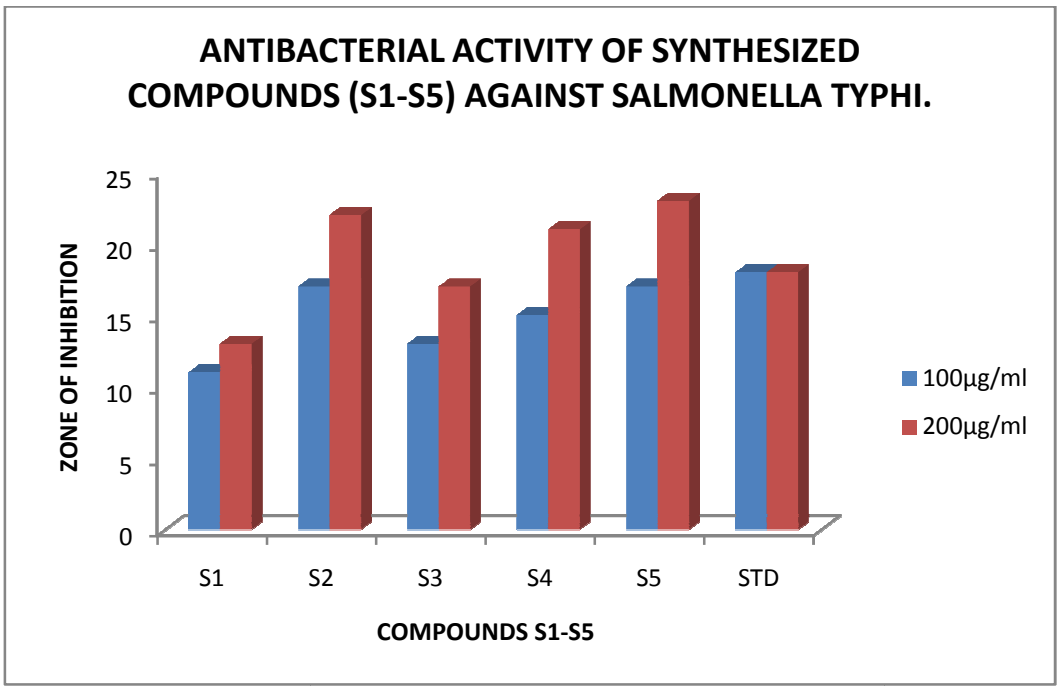


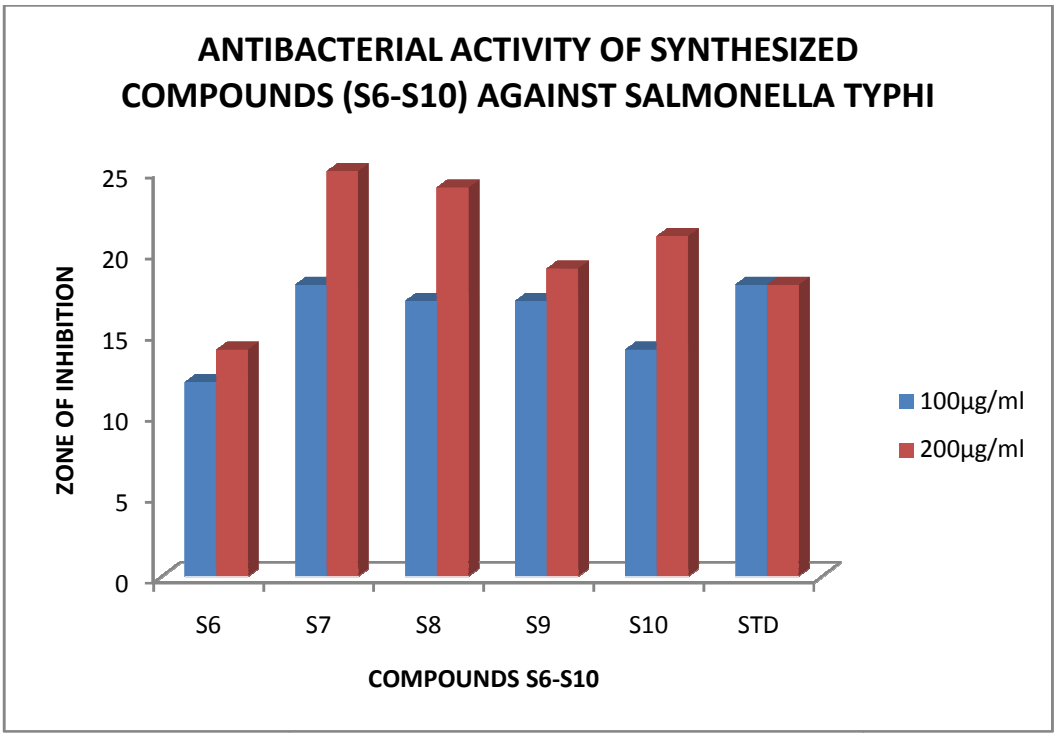


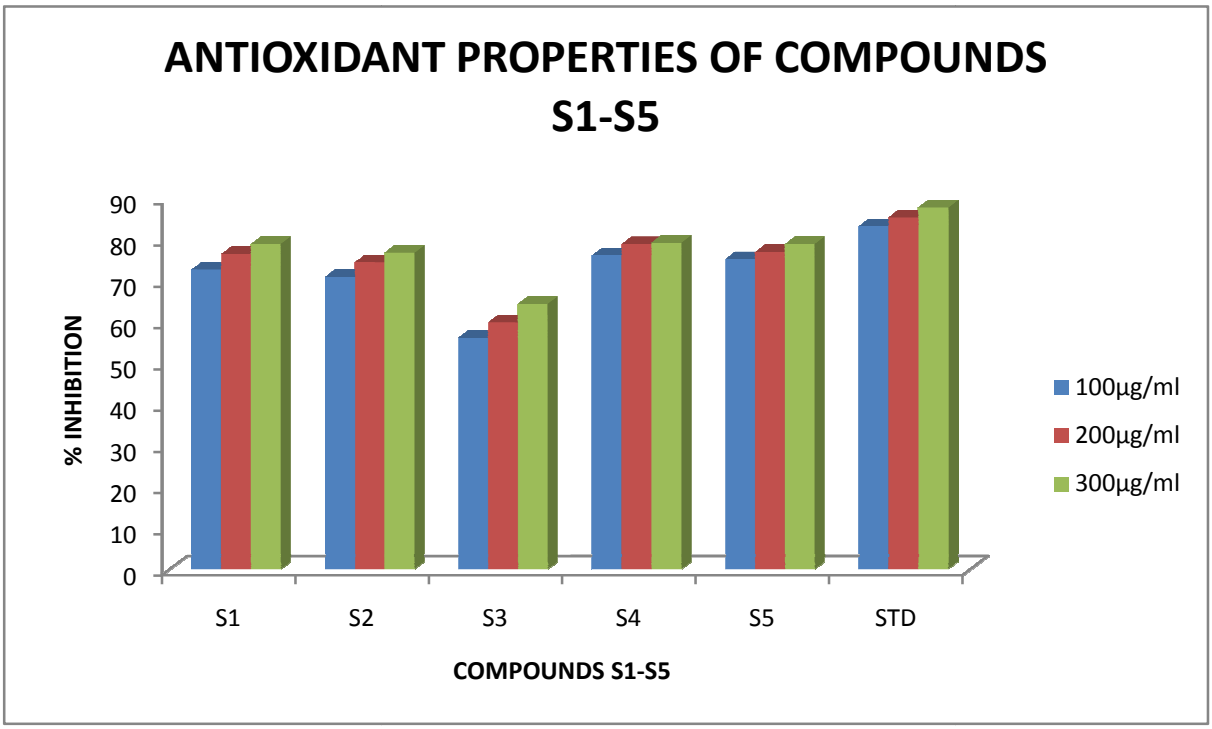

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS(S6-S10) AGAINST STAPHYLOCOCCUS AUREUS.
ZONE OF INHIBITION
25
20
15
10
5
0
S6
S7
S8
S9
S10
STD
COMPOUNDS S6-S10
100µg/ml
200µg/ml

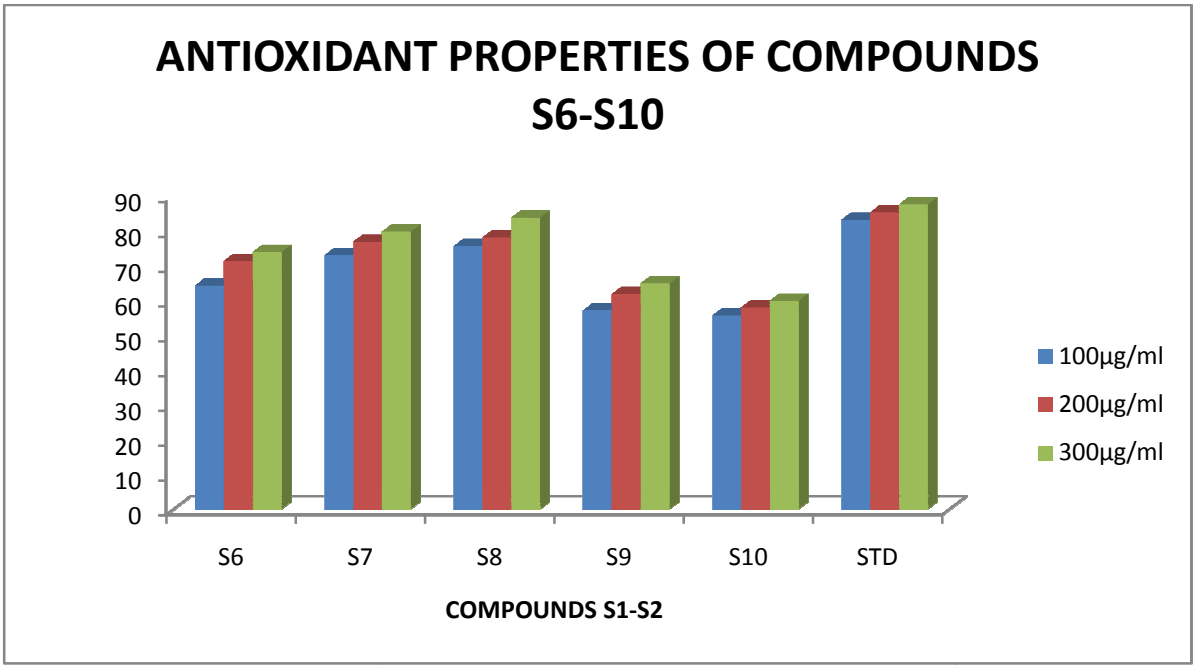

ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS(S6-S10) AGAINST STAPHYLOCOCCUS AUREUS.
ZONE OF INHIBITION
25
20
15
10
5
0
S6
S7
S8
S9
S10
STD
COMPOUNDS S6-S10
100µg/ml
200µg/ml

ANTI BACTERIAL ACTIVITY



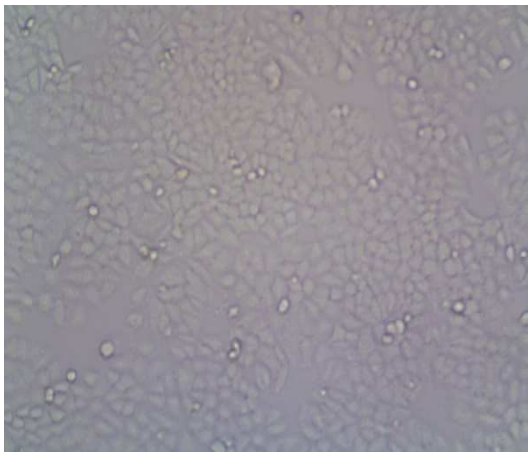






IN VITRO ANTI CANCER

COMPOUND S1(0.1µM)



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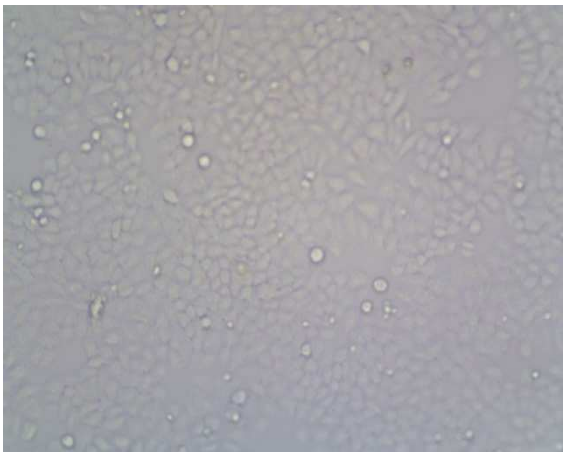

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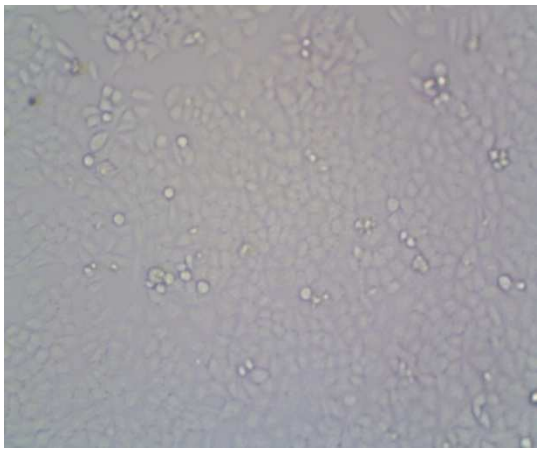

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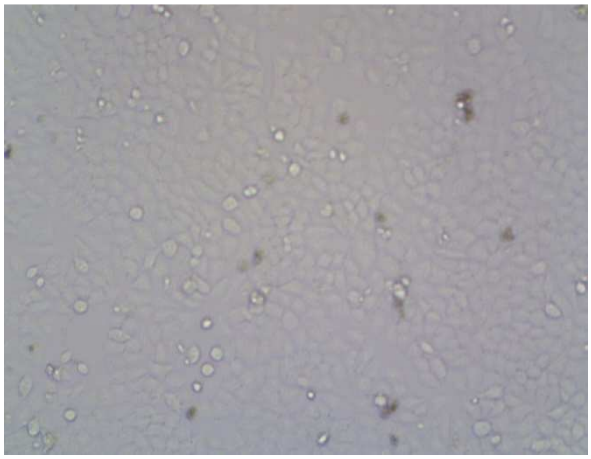

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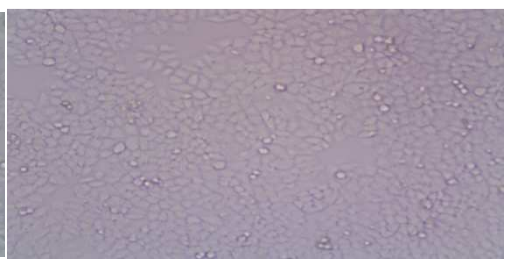

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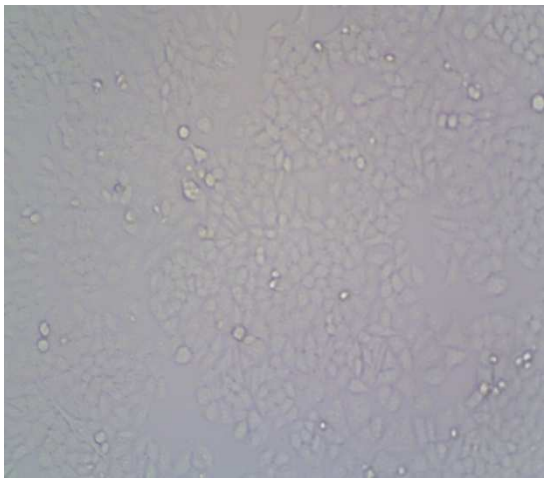

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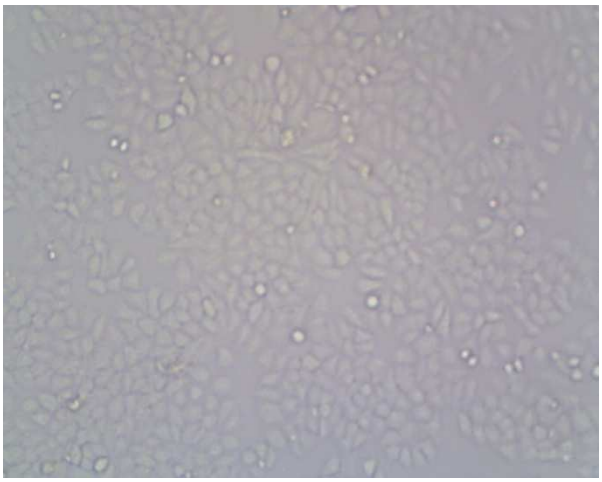

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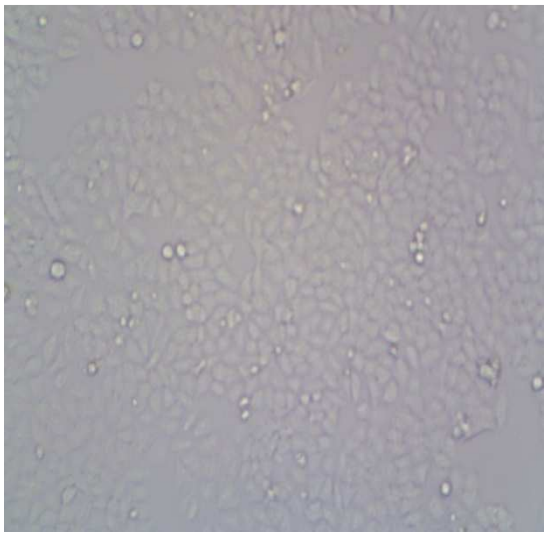

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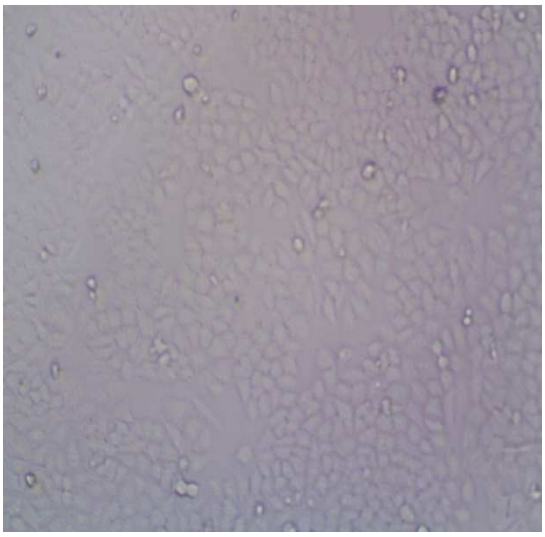

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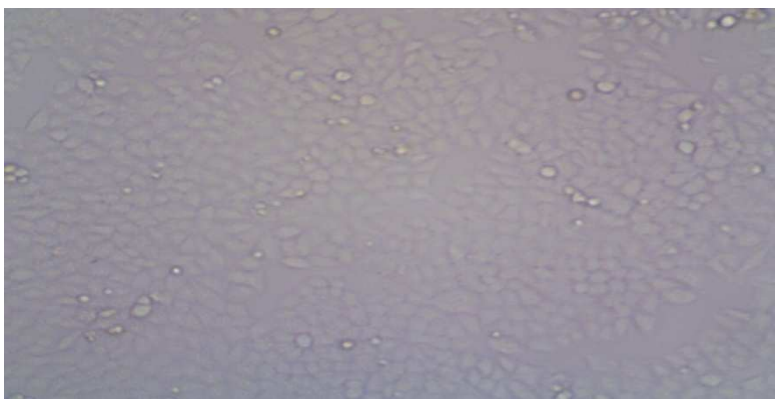

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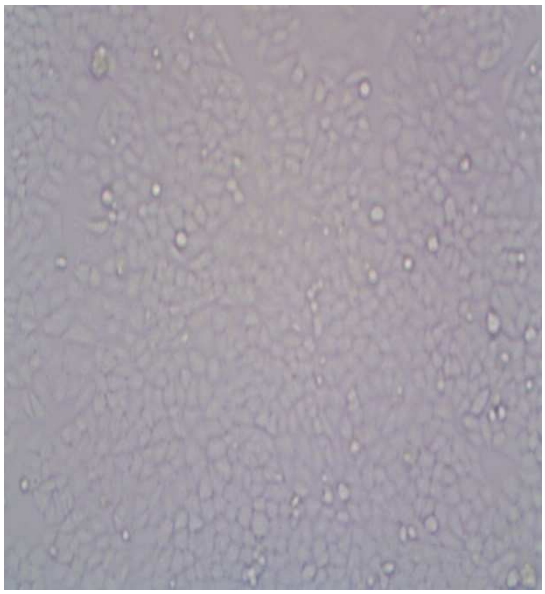

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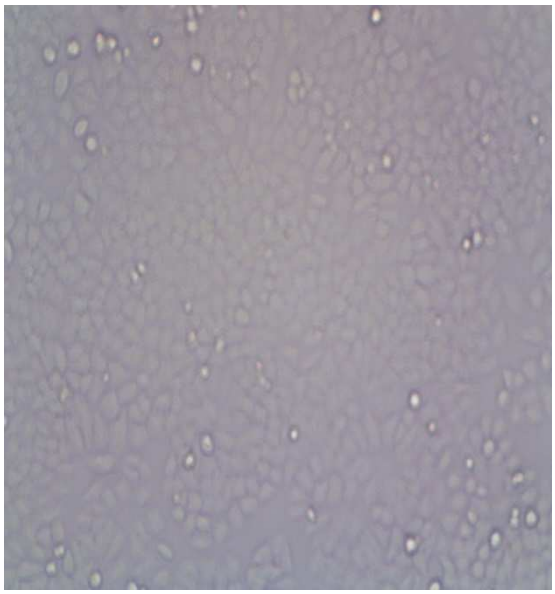

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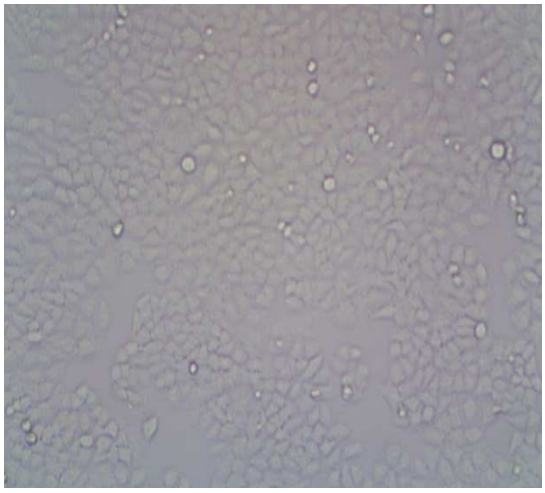

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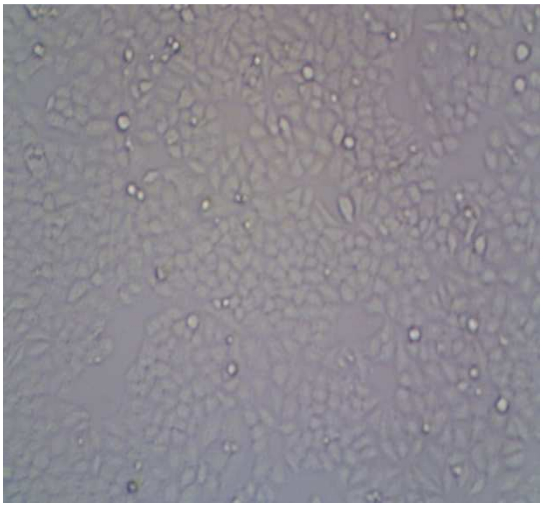

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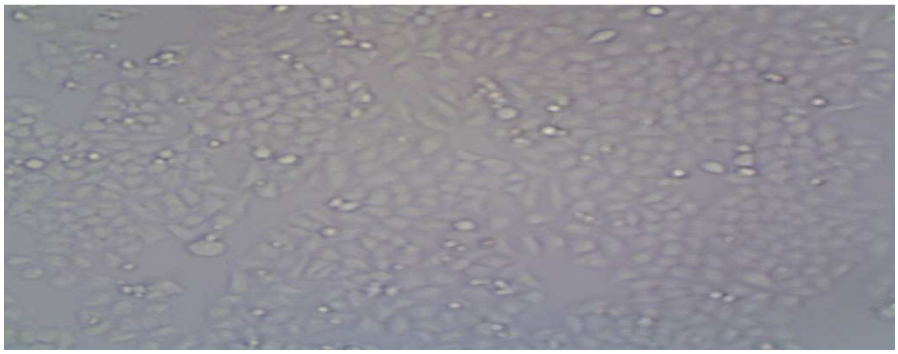

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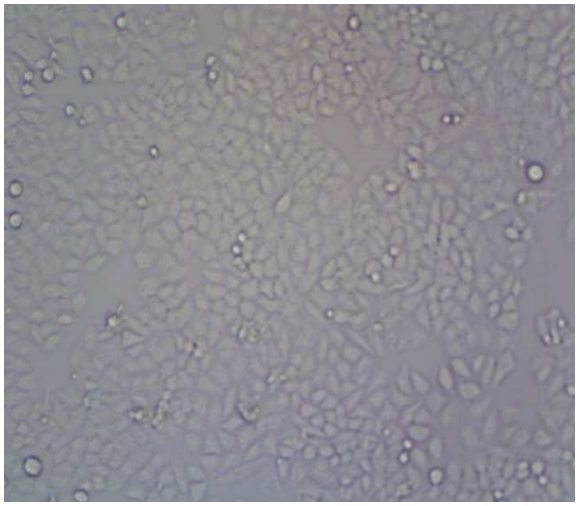

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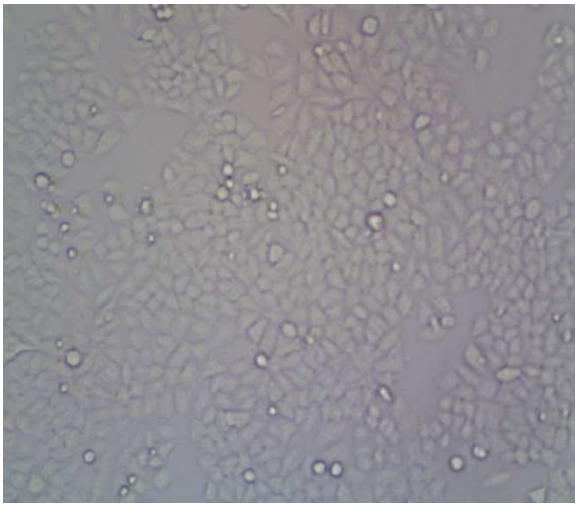

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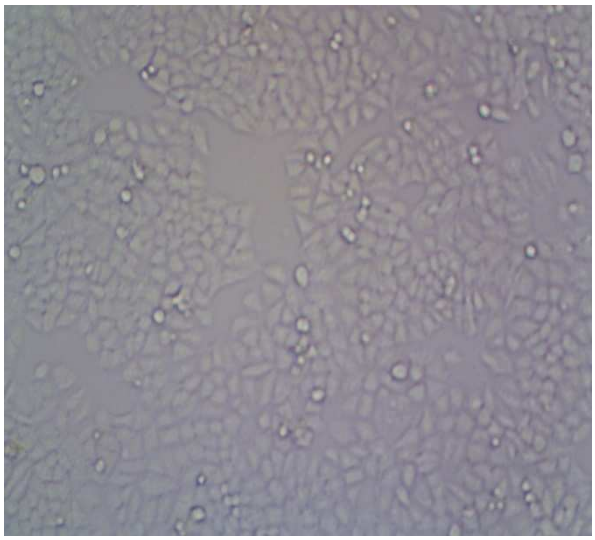

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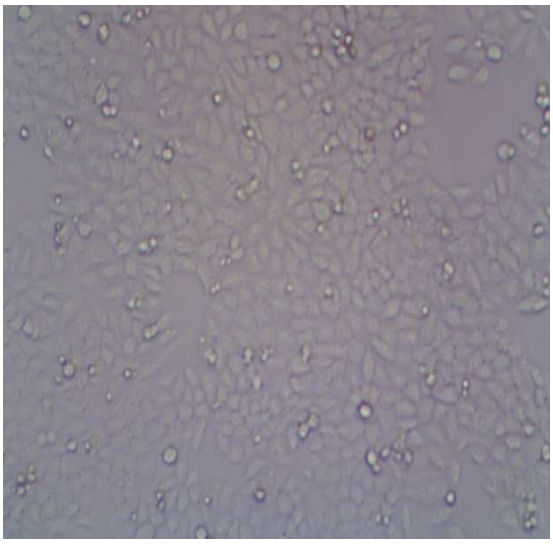

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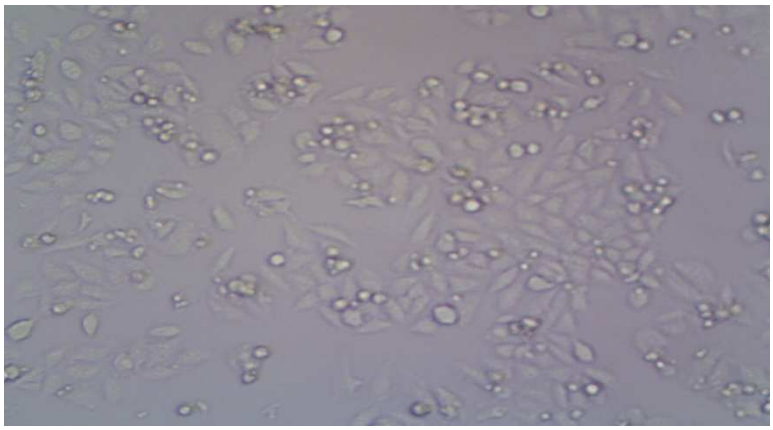
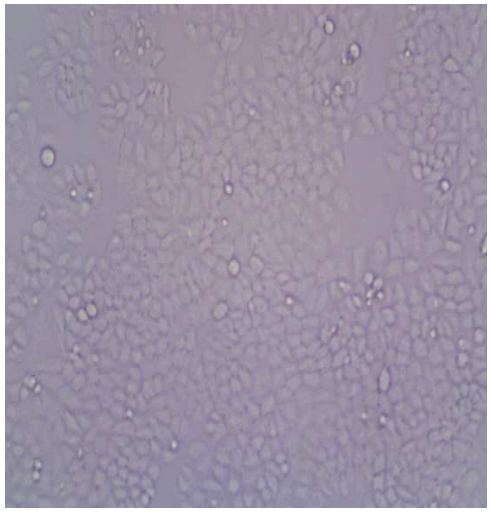
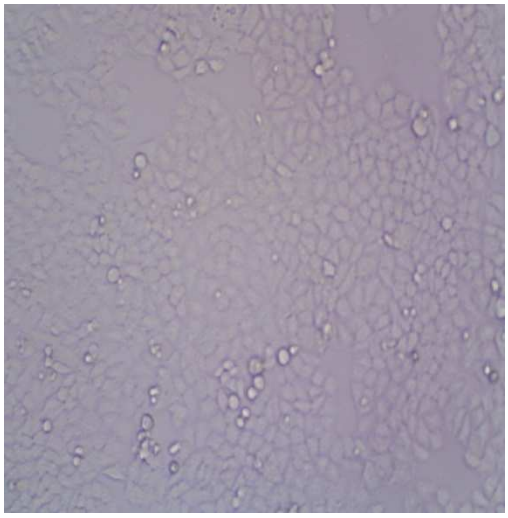


COMPOUND S9 (0.1µMl)

COMPOUND S9 (1µM)



COMPOUND S9(10µMl)



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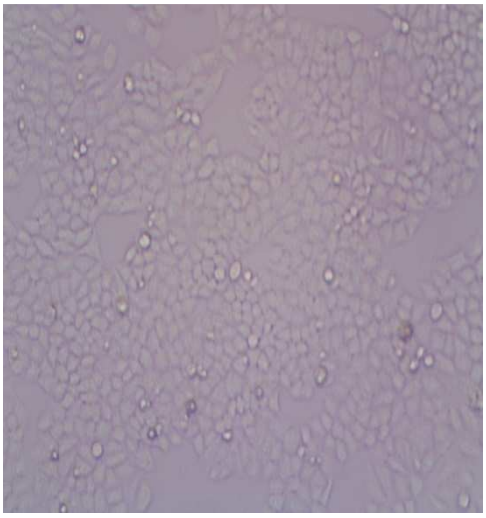

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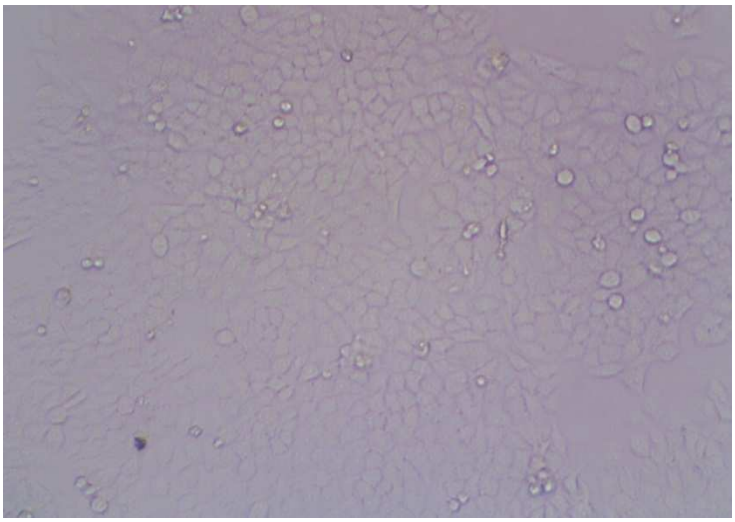
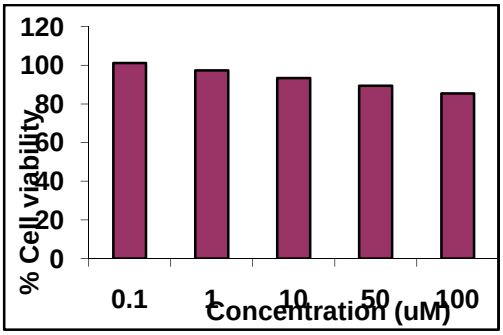


COMPOUND S1



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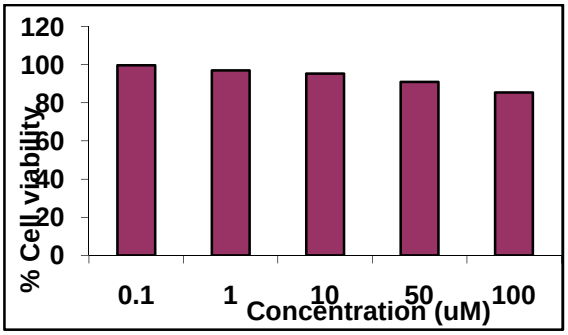

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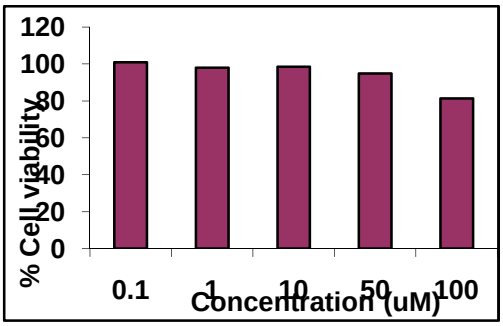

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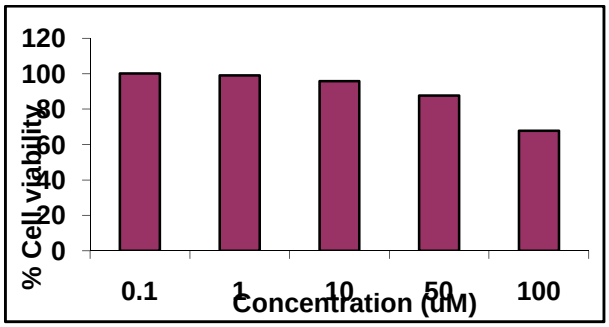

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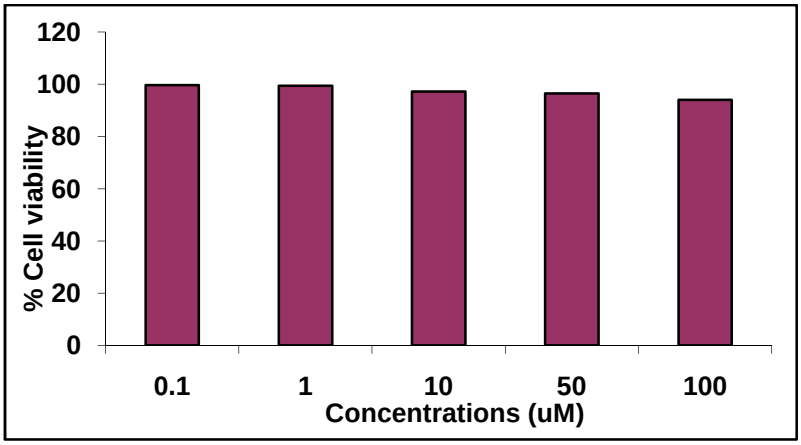

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